A Desk Study on Pesticide Metabolites, Degradation and Reaction Products to Inform the Inspectorate's Position on Monitoring Requirements

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Executive Summary

The Water Supply Regulations require that water from consumer taps should not have levels of individual pesticides and related products, including their relevant metabolites, degradation and reaction products that exceed 0.1 µg/L for individual pesticides, with the total sum of pesticide concentrations not to exceed 0.5 µg/L. Advice to water companies in the Inspectorate's Guidance Document is that "relevant" should be taken to mean any metabolites, degradation and reaction products that have similar pesticidal properties to their parent pesticides. Moreover general provisions of the Regulations require water companies to monitor for any substance that it believes may be present at a concentration that would constitute a potential danger to human health. The guidance document goes on to state that "in respect of drinking water, there is no evidence at the present time that any pesticide metabolites, degradation or reaction products are active pesticides or represent a risk to health and therefore no additional monitoring is required" Therefore the aims of the study were to review the area of pesticide metabolites, degradation and reaction products, to advise whether any additional monitoring needs to be conducted and to inform future guidance to the industry. These aims were met with six specific objectives:

- Review and collate information relating to pesticide metabolites, degradation and reaction products arising from pesticides (collectively termed metabolites in this study) currently approved for use in the UK;
- Identify metabolites that may exhibit pesticidal activity and/or may exhibit additional toxicological concerns;
- Estimate the likely concentrations of the metabolites identified in the previous objective in drinking water;
- Estimate the risk (if any) that these metabolites may pose to human health;
- Consider whether metabolites will react during water treatment with chlorine and ozone to form toxic compounds; and
- Advise whether the current guidance regarding pesticide metabolites needs to be updated.

The main findings of the project where:

- Limited data are available in the UK on the presence of pesticide metabolites in raw source and finished drinking waters. Most data available focus on metabolites of heptachlor and DDT with no data on metabolites from the majority of the high use pesticides;
- Data are available on the presence of pesticide metabolites in raw source and finished drinking waters for other countries, particularly the US but these data generally only focus on triazine and chloroacetamide herbicides;
- 485 metabolites were identified from pesticides with current approval in the UK and those that have recently lost approval;
- 53 metabolites were selected for further study; the selection was based on the potential to contaminate source water incorporating estimates of parental usage, formation rates in soil, persistence and mobility, and estimated toxicity and/or potential to exhibit pesticidal activity;
- Approximately half of the metabolites identified for further study are identified during environmental degradation as well as during mammalian degradation of the parent pesticides;
- Metabolite concentrations in raw source waters for three selected catchments were estimated using an empirical model previously developed to estimate pesticide concentrations on a catchment scale. A highly conservative estimate was generated and then refined on the basis of actual pesticide usage and/or more realistic metabolite soil DT_{50};
- Estimated removal efficiencies during water treatment of the selected metabolites ranged between 15 and 99%;
- Using computational method to predict the identity of compounds formed following chlorination and/or ozonation would have generated an excessive number of potential compounds making the further analysis extremely difficult;
- It was estimated that 21 metabolites would be detoxified by chlorination and/or ozonation, 11 were unlikely to be detoxified and due to a lack of information it was impossible to determine whether the remaining 21 metabolites studied would or would not be detoxified;
- Maximum concentration for any selected metabolite in finished drinking water using the more realistic estimate refined for actual pesticide usage and following conventional treatment (including powdered activated carbon) was 0.265 µg/L for metazachlor sulfonic acid a metabolite of the herbicide metazachlor which not considered to be pesticidally active;

- One of the metabolites selected on the basis of their potential pesticidal activity had an estimated concentration greater than 0.1 µg/L in finished drinking water for one of the catchments, phosphorous acid a metabolite of fosetyl-aluminium;
- Toxicological hazard assessments were performed for all selected metabolites and, in the absence of acceptable daily intake values, project specific derived values (PSDV) were estimated;
- Five metabolites had anticipated daily intakes greater than 10% of the acceptable daily intakes (or PSDV) for the conservative concentration estimate when considering toddlers; these fell below 10% when the refined estimate was considered;
- One metabolite had anticipated daily intakes greater than 10% of the acceptable daily intakes (or PSDV) for the conservative concentration estimate when considering adults; this fell below 10% when the refined estimate was considered; and
- None of the selected metabolites were considered to pose a significant risk to toddlers or adults.

Therefore considering the work undertaken in this study there is no evidence that the current guidance issued by DWI on the requirements to monitor for pesticide metabolites in drinking waters should be changed. However it may be prudent to validate this conclusion with experimental data due to the uncertainty that can be associated with predictive approaches.

Contents

1 Introduction

Pesticide application in agriculture is used to increase crop yield and maintain plant health by eradicating unwanted organisms that compete for resources, bestow disease and/or cause crop damage due to feeding activities. During a normal growing season a wide variety of pesticides can be applied and their identity depends on a range of factors including the specific pest and crop of interest. Once released into the environment, the pesticide is susceptible to biological and chemical degradation, which may result in the formation of a range of different compounds, commonly termed 'metabolites'. Once formed in the environment, metabolites can move vertically through the soil profile to underlying groundwaters and away from the site of application via aquifer transport. There is also the potential for metabolites to enter surface waters when they travel laterally via either overland runoff due to heavy rainfall or via sub-soil tile drains, entering agricultural ditches and streams and are then transported on to major rivers, reservoirs and ultimately estuaries and the marine environment.

Some pesticide metabolites have been identified in rivers, reservoirs and groundwaters throughout the world. There is therefore the potential that raw water abstracted for treatment and subsequent human consumption may contain trace concentrations of these compounds together with their parent pesticides. Current regulations require that water at consumers taps does not have levels of individual pesticides and related products, including their relevant metabolites, degradation and reaction products that exceed 0.1 µg/L (apart from aldrin, dieldrin, heptachlor and heptachlor epoxide which have compound specific levels set at 0.03 µg/L). In addition with the total sum of detectable pesticide concentrations, termed total pesticides, must not to exceed 0.5 µg/L (The Water Supply (Water Quality) Regulations 2000).

It is generally accepted that trace concentrations of pesticides in drinking water are far smaller than those known to be harmful or likely to cause harm and there is good reason to suppose that, in general, concentrations of metabolites are unlikely to be significantly higher than parent pesticide concentrations. The aim of this project was to investigate whether these anticipated low level concentrations of pesticide metabolite could exceed the pesticide standard or pose a risk to the UK population through consumption of drinking water. This aim was achieved through six clear objectives:

1. Review and collate identity, property and occurrence information relating to pesticide metabolites arising from pesticides currently approved for use in the UK;
2. Identify metabolites that may exhibit pesticidal activity and/or may exhibit additional toxicological concerns and select some for further study;
3. Perform environmental fate modelling to estimate catchment level concentrations and to refine these concentrations with water treatment removal efficiencies to estimate the likely concentrations of selected metabolites in finished drinking waters;
4. Determine the risks to the UK population posed by selected metabolites; and
5. Investigate the hazard that could be posed by metabolite byproducts formed during by chlorination and/or ozonation water treatments.

2 Metabolite Data Collation

2.1 Occurrence of Metabolites in Source and Treated Drinking Waters

2.1.1 UK specific data

Data was sought on the reported concentrations of pesticide metabolites in source and treated drinking waters within the UK. This focused on compounds that can be considered conventional metabolites, i.e. formed due to pesticide biotic or abiotic degradation following application. Data was not sought on compounds that can be both conventional metabolites and also pesticides in their own right since it would be difficult to separate the different contributions to the levels determined in waters.

The Environment Agency (EA) and water companies were contacted and any available data they held concerning the detection of pesticide metabolites in raw and treated waters were collated. The data provided by the EA provides details of pesticide metabolite analysis undertaken between January 2003 and December 2007. Extensive datasets were provided which contain ~50,000 analyses undertaken for surface water and ~44,000 analyses for groundwaters. Summaries of these extensive datasets can be found in Appendix 1 (surface water) and Appendix 2 (groundwater). DWI provided a list of water company contacts and these details were used to make a request for data relating to the analysis for pesticide metabolites in raw and treated drinking waters. This request was made by email and 27 companies were contacted of which 6 companies did not respond to the request, 12 companies replied that they had no data available that would benefit the project and 9 companies provided data. These data are summarised in Appendix 3. A summary of the maximum concentrations is provided in Table 1.

Table 1. Summary of EA and water company data on pesticide metabolite concentrations in environmental/raw water and treated/finished water

Metabolite	Parent Pesticide	Maximum concentration (µg/L)	
		Environmental and/or raw water	Finished/treated water
deethylatrazine	atrazine	1.02	0.0575
deisopropylatrazine	atrazine	0.914	0.0124
op-DDE	DDT	0.01	0.004
op-TDE	DDT	0.02	0.004
pp-DDE	DDT	0.016	0.004
pp-TDE	DDT	0.012	0.006
heptachlor epoxide	heptachlor	0.0133	0.01

The most prevalent data from these sources related to the detection of metabolites from the organochlorine insecticides, DDT and heptachlor, i.e. TDE, DDE and heptachlor epoxide.

The limited monitoring of pesticide metabolites by water companies can be attributed to the current regulations and associated Guidance. Within the regulations a standard of 0.03 µg/L is set for heptachlor epoxide, the only specifically identified metabolite and 0.5 µg/L for "total pesticides" which is determined as "the sum of the detected concentrations of the individual pesticides and any relevant metabolites, degradation and reaction products detected and quantified in the samples taken on a particular sampling occasion from a sampling point". However the Guidance does go on to state that "relevant should be taken to mean any metabolites, degradation and reaction products that have similar pesticidal properties to their parent pesticides" and "DWI considers that, in respect of drinking water, there is no evidence at the present time that any pesticide metabolites, degradation or reaction products are active pesticides or represent a risk to health and therefore no additional monitoring is required".

2.1.2 Occurrence data from other countries

Metabolite environmental occurrence data collated from the scientific literature for environmental waters have been collated and are provided in Appendix 4. No additional data could be identified in the scientific literature for the occurrence of pesticide metabolites in UK environmental waters. Data sources searched included the scientific literature through the Web of Knowledge portal and pesticide regulatory review documents produced the UK Pesticide Safety Directorate, US Environmental Protection Agency, European Food Safety Authority and Canadian Pest Management Regulatory Agency. The majority of the identified data is for the US focussing on the identification of herbicide metabolites from the triazine and chloroacetamide chemical classes.

2.2 Identification of Metabolites

2.2.1 Pesticides approved for use in the Great Britain

Using the data and databases collated and maintained by the Fera Pesticide Usage Survey Team (PUS) a list of 276 pesticide active ingredients currently approved for

use in Great Britain was generated and provided to the project team (list generated 31st August 2008). Collated together with this list of approved active ingredients were the most recent pesticide usage estimates for Great Britain. Data were from both LIAISON (2008) (a Fera database of pesticide approvals, manufacturers' labels and manuals) and the pesticide usage statistics.

The PUS have experienced surveyors who make personal visits to holdings across England and Wales. Similar data for Scotland are also collated at Fera to provide information for Great Britain as a whole. All holdings are selected from a random sample, stratified by holding size and region. The information is collected on a field by field basis for each crop and is then raised using data from the annual agricultural census returns to give national estimates of usage. The PUS has been collating pesticide usage estimates more than 40 years but since the introduction of the Food and Environment Protection Act in 1985, the post registration monitoring of pesticides became a legal requirement.

The usage estimates supplied to the project were the most recent available (2006) and covered all agricultural and horticultural crops grown commercially throughout Great Britain. The data included protected crops, such as mushrooms and glasshouse crops, but excluded amateur and amenity use and use on hard surfaces and non-agricultural areas, such as roads, railways, pavements and areas around utilities which can be a significant input of pesticide residues to surface waters (e.g. Ramwell et al. 2002). Figure 2.1 provides a representation of the pesticide estimates of usage provided.

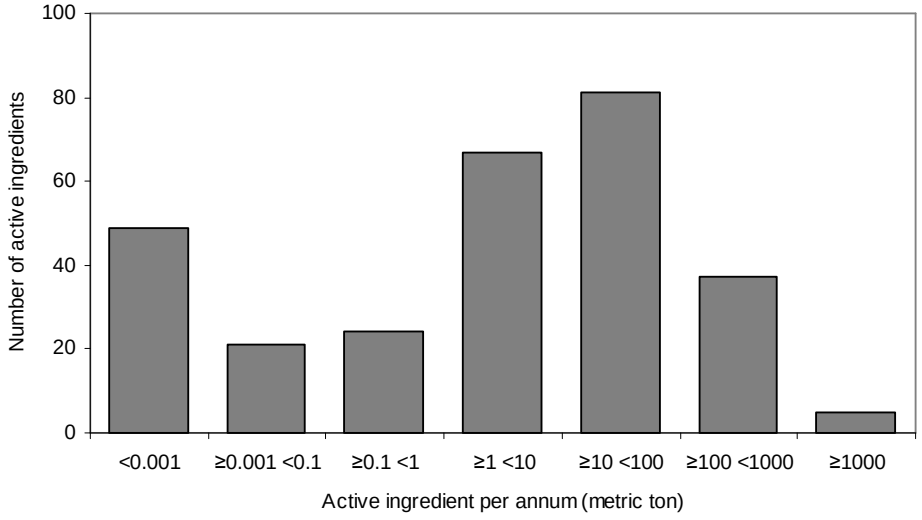

Figure 2.1. Pesticide usage in Great Britain 2006

The list of active ingredients approved for use in the UK was reviewed and any compounds present on the list considered to be inorganic, e.g. sulphuric acid, biological, e.g. *Bacillus thuringiensis*, and/or had an undefined chemistry, e.g. fatty acids were removed (n = 23).

A number of active ingredients (n = 36) were estimated to have very low annual usage (< 1 kg), two reasons were identified:

- The active ingredients were used very little in Great Britain, e.g. maneb; or
- The active ingredients were too new to have been encountered during the last rounds of surveying, e.g. aminopyralid.

Those compounds with limited usage estimates because the active ingredients have only recently gained approval and were therefore not encountered during the last usage surveying were identified (n = 22). The remainder are considered 'old' compounds with current approval but are not used enough to be encountered during usage surveying (n = 14).

Moreover the LIAISON database was also interrogated for information on pesticides that have lost UK approval over the past three years. It was considered that metabolites from these pesticides may still pose a risk to drinking water if they are persistent in the environment. Thirty-three pesticides were identified as losing UK approval in the past three years. Therefore 286 active ingredients were considered during following project objectives.

2.2.2 Metabolites identified from pesticides

Metabolites identified in aerobic soil degradation studies were identified for the pesticides. This was not possible for all pesticides approved for use in the UK since:

1, Some pesticides had no (main/major) metabolites identified in aerobic soil degradation studies (n = 25); or
2, Some pesticides do not have appropriate aerobic soil degradation study data available (n = 76).

Leaving 185 pesticides for which metabolites could be identified. The majority of metabolite formation data in aerobic soil degradation studies were collated from the regulatory review documents for pesticides (PSD, EPA, EFSA, EU, PMRA). Where detailed pesticide review documents were not available or the review did not identify soil metabolites, the pesticide degradation compendiums of Roberts (1998) and Roberts and Hutson (1999) were used to identify the structures of primary soil metabolites (n = 21 pesticides). Maximum formation rate, i.e. the highest metabolite concentration formed expressed as a percentage of the initial active ingredient, and time to maximum formation were noted from these studies. If multiple studies were available then the maximum value from all studies was collated.

In total 523 soil metabolites were identified from 185 pesticides. Within the literature, metabolites could be defined by a number of means, e.g. common name, two-dimensional structure, IUPAC name, CAS number and/or manufacturers code. In some cases metabolite definition in the literature was unsuitable for identification of two-dimensional structure e.g. when only defined by manufacturers code. Therefore of the 523 metabolites 2-dimensional structural representations could be identified for 485 (92.7%). Estimates of mammalian toxicity using DEREK and TOPKAT and potential pesticidal activity through presence of toxicophores were then undertaken for the 485 metabolites and their parent pesticides.

3 Toxicity and pesticidal activity

3.1 Toxicity

The chemical structures of the metabolites were drawn in ChemDraw Ultra version 10.0 (CambridgeSoft Corporation). These two dimensional structures were saved in both .cdx and .mol file formats for future use. Two-dimensional structures, in .cdx file format, were imported into Accord of Excel version 6.1 (Accelrys Inc.). Accord was used to generate SMILES (Simplified Molecular Input Line Entry System) notations for each of the study compounds from the two-dimensional structures. Metabolite mammalian toxicity using this structural data was estimated using two approaches, namely DEREK and TOPKAT.

3.1.1 DEREK

Metabolites were evaluated using the expert software DEREK for Windows version 10.0.2 (Lhasa Ltd). DEREK works by matching structural entities in a query structure with structural alerts that are associated with different toxicity endpoints (toxicophores). A structure alert is the set of structural features in a molecule that suggests to a toxicologist that the substance may show a particular toxic effect. This is similar to the definition of a toxicophore (a structural feature believed to be responsible for its toxic effect) and a pharmacophore (a structural feature believed to be responsible for a useful pharmacological effect), but alerts and toxicophores are not always identical. An alert may include information about additional features that increase or decrease the effectiveness of a toxicophore. A full list of endpoints assessed by DEREK is given in Appendix 5. In total the program uses ~500 structural alerts associated with different endpoints.

DEREK identifies alerts for the following toxicity endpoints for species that include bacteria (Salmonella typhimurium), guinea pig, hamster, human, mammal, mouse, primate, rat and rodent:

- Carcinogenicity, such as photocarcinogenicity
- Irritation, such as irritation of the eye and the gastrointestinal tract
- Miscellaneous endpoints, such as anaphylaxis and anticholinesterase activity
- Genotoxicity, such as mutagenicity and chromosome damage
- Respiratory sensitisation, such as occupational asthma
- Skin sensitisation, such as photoallergenicity

- Thyroid toxicity

DEREK provides an indication of the likelihood of each predicted adverse effect using the terminology shown in Table 2.

Table 2. Terminology used in the outputs provided by DEREK for each endpoint

Terminology	Description
Certain	There is proof that the proposition is true
Probable	There is at least one strong argument that the proposition is true and there are no arguments against it
Plausible	The weight of evidence supports the proposition
Equivocal	There is an equal weight of evidence for and against the proposition
Doubted	The weight of evidence opposes the proposition
Improbable	There is at least one strong argument that the proposition is false and there are no arguments that it is true
Impossible	There is proof that the proposition is false
Open	There is no evidence that supports or opposes the proposition
Contradicted	There is proof both that the proposition is true and that it is false

DEREK estimations were considered to provide a positive response for an end-point when an alert for that end-point was identified within a query molecule and that estimate was categorised as at least ‘plausible’. DEREK was assumed to estimate a negative response for the end-point of interest in no structural alerts for that end-point were identified. A summary of the alerts identified from the 485 metabolites are provided in Table 3.

Table 3. Summary of the estimates generated by DEREK for the 485 metabolites

End-point identified	Number of metabolites
Nothing to report	158
Carcinogenicity [a]	***188***
Skin sensitisation	145
Hepatotoxicity	75
Mutagenicity [a]	***72***
Chromosome damage	52
Bladder urothelial hyperplasia	31
Ocular toxicity	24
Cholinesterase inhibition	21
Methaemoglobinaemia	17
Phototoxicity	14
Nephrotoxicity	13
Teratogenicity [b]	***13***
Cyanide-type effects	9
Irritation	8
Thyroid toxicity [b]	***8***
Genotoxicity	7
Respiratory sensitisation	7
Chlorance	5
Neurotoxicity	3
Photoallergenicity	2
Uncoupler of oxidative phosphorylation	2
HERG channel inhibition	1
Pulmonary toxicity	1

[a] – End-points identified that should be considered for metabolites in drinking water

[b] – Additional end-points identified by IEH that should also be considered

3.1.2 TOPKAT

The mol files generated using ChemDraw were used to predict the potential toxicity of the metabolites using the models and relevant sub-models contained within TOPKAT, a module within Discovery Studio version 1.7 (Accelrys Inc.). TOPKAT contains a range of robust, cross-validated Quantitative Structure Activity Relationships (QSAR), which are multivariate statistical relationships between experimentally derived toxicity data and chemical descriptors. When a compound is entered into TOPKAT the chemical descriptors are derived from the input structure. These descriptors quantify chemical transport properties and the biochemical interaction with the target site. The predictions of toxicity are derived using the chemical descriptor values for the compound of interest and the multivariate statistical relationships. TOPKAT is also able to determine whether the compound of interest falls within the applicability domain of the model/relationship. This is determined using the 'optimum prediction space' (OPS) and is a unique multivariate descriptor space in which the model is applicable (Accelrys Inc., 2004). The following toxicity end-points available in TOPKAT were estimated:

- Developmental toxicity potential
- Mutagenicity (Ames test)
- Rodent carcinogenicity
- Rat oral LD50

Predictions were only considered if they fell within the optimum prediction space and all validation criteria were satisfied, or if they fell outside the optimum prediction space but within a permissible range (as determined by TOPKAT). Results were not considered for end-points where the program identifies that a prediction may be unreliable because either:

- the prediction was outside the optimum prediction space and outside the permissible range of the model/sub-model or,
- a structural fragment from the query compound was not represented in the training set of the model/sub-model.

A summary of the applicability domain results provided by TOPKAT is provided in Table 4. Of those estimates considered reliable, i.e. within the applicability domain of the respective model, a summary of the estimates generated by TOPKAT are provided in Table 5.

Table 4. Applicability domain results for the four models used within TOPKAT for the 485 metabolites

Model	Within OPS [b]	Outside OPS but within OPS limits [b]	Outside OPS and OPS limits	Estimate not possible [a]	Total
Developmental toxicity potential model	341	63	80	1	485
Mutagenicity (Ames test)	373	19	92	1	485
Rodent carcinogenicity	389	33	62	1	485
Rat oral LD50	386	28	70	1	485

[a] – TOPKAT classed one metabolite as inorganic and would not provide an estimate for this molecule using any model.

[b] – Criteria for which estimates are classified to be within applicability domain of the model and therefore estimate can be considered reliable

Table 5. Summary of the toxicity estimates generated by TOPKAT for the metabolites

Model/estimate		Number of metabolites
Rat oral LD50 [b]		
High toxicity	(1-50 mg/kg bw)	32
Moderate toxicity	(50-500 mg/kg bw)	95
Low toxicity	(50-500 mg/kg bw)	215
Very low toxicity	(500-5000 mg/kg bw)	72
Developmental toxicity potential model		
Developmental toxicant		148
Non-developmental toxicant		217
Indeterminate [a]		39
Mutagenicity (Ames test)		
Mutangen		65
Non-mutagen		315
Indeterminate [a]		9
Rodent carcinogenicity		
Carcinogen		144
Non-carcinogen		261
Indeterminate [a]		17

[a] – An estimate is considered indeterminate when the estimated probability of a compound is too close to chance, i.e. 50%. Estimates are considered indeterminate if the estimated probability was >30% and <70%.

[b] – Classification (in mg/kg body weight) according to the Hodge and Sterner scale (1949), no metabolites were estimated to be exhibit 'extremely high toxicity' or be 'relatively harmless', the two additional classes on this scale.

3.1.3 Limitations of the predictive tools

Predictive tools such as those employed during this project do have limitations which are important to consider whenever evaluating/utilising their output:

- DEREK is an expert system approach based on structural fragments previously identified in the literature, therefore it is highly probable that not all fragments that cause a specific effect are present within its structural alert dataset;
- DEREK and TOPKAT were not specifically developed to estimate the toxicity of pesticides (and their metabolites) and therefore these types of chemicals may be under-represented within the training sets used to develop the approaches;
- For some end-points, e.g. mutagenic response in an Ames test, inter-laboratory concordance of experimental studies rarely exceeds 80-90% therefore predictive methodologies cannot be expected to perform better than the experimental work they are based on;
- The applicability domain is an important concept to consider during the use of predictive approaches. One of the ways of ensuring an estimate is valid is to ensure that the query molecule fits within the chemical descriptor space

described when the model was developed. Generally not all query molecules, e.g. metabolites in this project, will fit within the applicability domain of models, therefore valid estimates cannot be generated for all endpoints for all compounds;

- The predictive ability of these approaches to estimate mutagenicity and rat oral LD_{50} have been evaluated specifically for pesticide metabolites (Sinclair 2009) How these approaches perform for other end-points when used for metabolites is unknown, but there are no particular reasons why the performance should be too dissimilar to other chemical classes/categories; and
- Predicative approaches are based on known toxicological data and are therefore unable to provide information on toxicological effects not previously identified experimentally.

However in the absence of experimental toxicological data on metabolites, predictive tools are all that can be used.

3.2 Pesticidal activity

It is important to identify whether a metabolite exhibits the same mode of action as its parent pesticide or not. Ignoring pro-pesticides, it can be generally assumed that the potency of a metabolite with the same mode of action as the parent pesticide, will be lower, since, during the commercial identification of potential active ingredients, the metabolite would have been selected rather than the parent pesticide. A reduction in potency could be due to a variety of reasons which may include changes in physico-chemical properties, persistence, molecular hindrance etc, all of which could impact the amount of the compound that reaches the site of action.

Without a comprehensive toxicity and ecotoxicity dataset for a range of target and non-target organisms, which is generally absent for the large majority of metabolites, it is difficult to make the decision whether a metabolite does exhibit the same mode of action on the basis of experimental data. Therefore during this study the determination on whether a metabolite may exhibit the same mode of action as the parent pesticide was performed using information available in the literature concerning the presence of the structural fragment previously attributed to exhibiting the mode of action for the chemical class of the parent pesticide. The two-dimensional structures of the metabolites were examined for the presence of these

structural fragments. This approach uses the premise that the exhibition of a specific mode of action is related to the interaction between a structural fragment of the molecule, commonly called the toxicophore, and the site of action within the effected organism (Escher and Hermens 2002). Therefore it is assumed that if, during the degradation process, the metabolite has lost the structural fragment attributed with exhibiting the mode of action, then the metabolite has lost the ability to exhibit that mode of action.

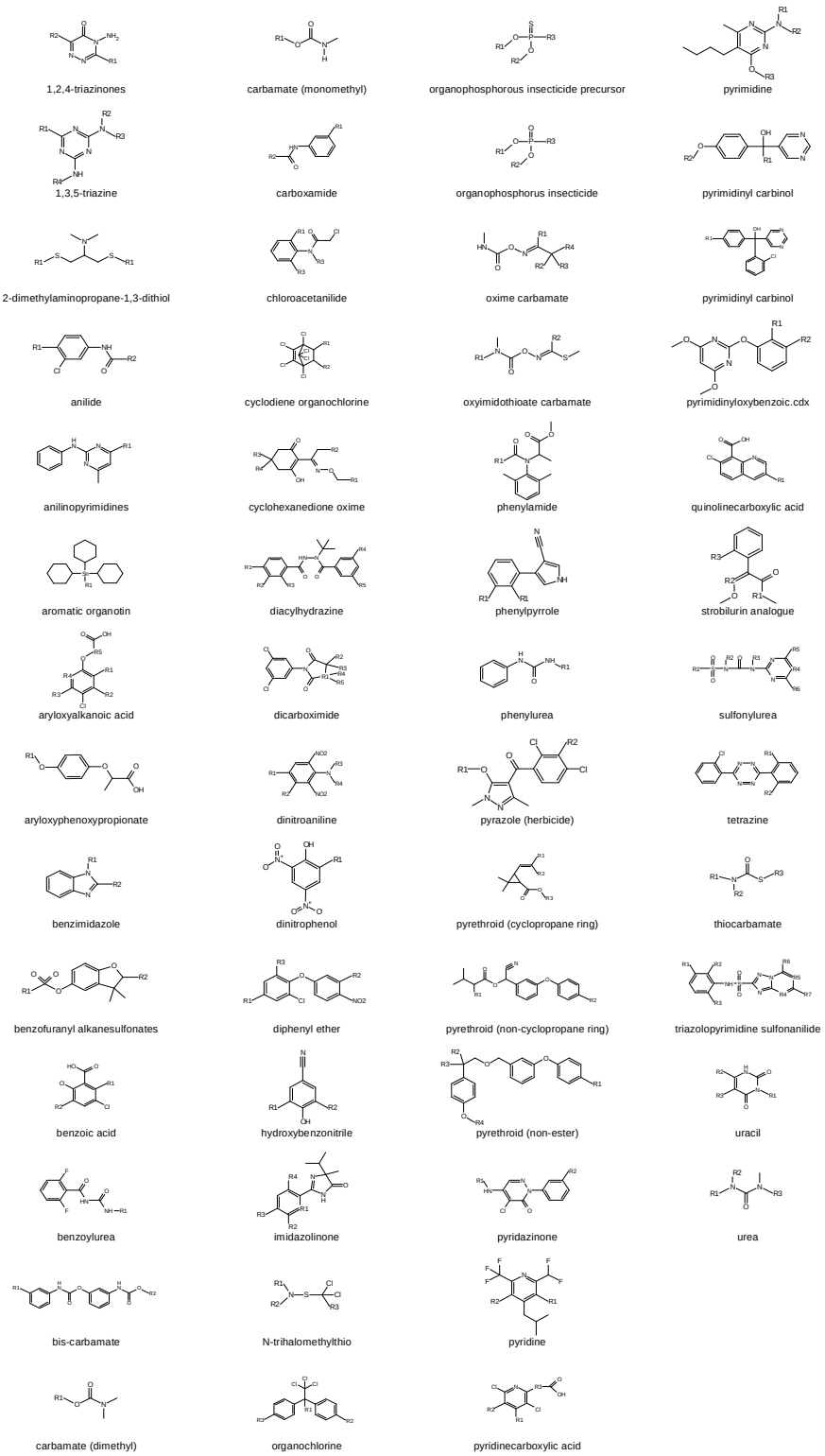

Figure 3.1. Common sub-structures identified for pesticide chemical classes (from Sinclair and Boxall, 2003).

Initially the 485 metabolite structures were examined for structural fragments previously identified as being present in pesticides attributed to certain pesticidal chemical classes (Figure 3.1). These structural fragments were developed by identifying the commonality within pesticidal chemical classes, e.g. a fragment that can be described as a sulfonylurea bridge joined to a least one aromatic ring, usually a triazine or diazine, is common to all sulfonylurea herbicides (Sinclair and Boxall 2003). Whilst these structures are probably smaller than the true toxicophore we can assume that if this sub-structure has been disrupted during degradation then the metabolite will not be capable of exhibiting the mode of action attributed to the parent pesticide. This analysis generated four responses: 1, the parent toxicophore was unaltered and therefore intact within the structure of the metabolite; 2, the parent toxicophore has been disrupted during degradation and therefore it is unlikely the metabolite will exhibit the specific mode of action; 3, No toxicophore was available for the parental pesticide chemical class from which the metabolite is formed; and 4, the parental pesticide was not attributed to a specific pesticidal chemical class. A summary of the number of metabolites which met each of these responses is provided in Table 6.

Table 6. Analysis of metabolite structures for the presence of sub-structural moieties provided in Figure 3.1

Response	Number of metabolites
Toxicophore is present in metabolite	107
Toxicophore is not present in metabolite	184
No toxicophore available for the pesticide chemical class	76
Pesticide not attributed to a chemical class	118

Therefore based on this initial assessment we can conclude that 184 metabolites do not contain the structure common to all pesticides from the parental chemical class and therefore will probably not exhibit the mode of action of the parent pesticide. A further investigation into those metabolites that either had the parental toxicophore still present in their structure or had no toxicophore information available was undertaken. A number of literature sources that examine the mode of action of pesticides where used for this purpose (e.g. Copping and Hewitt 1998; Roberts 1998; Roberts and Hutson 1999; Tomlin 2000; Cremlyn 1991; Corbett et al. 1984). These texts were used to determine whether any further information can be identified on the exhibition of parental pesticidal toxicity by metabolites that either had a toxicophore

retained in their structure, had no toxicophore available for the chemical class of the parent pesticide, or the parent pesticide is not attributed to a chemical class with an identified toxicophore. Results of this analysis are presented in Table 7.

Table 7. Results from the further investigation of metabolites exhibiting pesticidal activity

Response		Number of metabolites
Toxicophore is present in metabolite		107
	There is evidence that the metabolite will not exhibit the parent pesticide mode of action	34
	No additional data is available to contradict that the metabolite could exhibit the pesticide mode of action based on the presence of the toxicophore	16
	Additional data was not conclusive to contradict that the metabolite could exhibit the pesticide mode of action based on the presence of the toxicophore	21
	There is evidence that the metabolite will exhibit pesticidal activity since the parent/metabolite act as a pro-pesticide	10
	There is further evidence that the metabolite will exhibit the parent pesticidal mode of action	23
	The metabolite will exhibit pesticidal activity since it is a commercial pesticide in its own right	3
No toxicophore available for the pesticide chemical class		76
	There is evidence that the metabolite will not exhibit the parent pesticide mode of action	10
	No additional data is available to determine whether the metabolite exhibits the pesticide mode of action	47
	Additional data was not conclusive to determine whether the metabolite exhibits the pesticide mode of action	16
	There is evidence that the metabolite will exhibit the parent pesticidal mode of action	3
Pesticide not attributed to a chemical class		118
	There is evidence that the metabolite will not exhibit the parent pesticide mode of action	9
	No additional data is available to determine whether the metabolite exhibits the pesticide mode of action	52
	Additional data was not conclusive to determine is available to determine whether the metabolite exhibits the pesticide mode of action	51
	There is evidence that the metabolite will exhibit pesticidal activity since the parent/metabolite act as a pro-pesticide	2
	There is evidence that the metabolite will exhibit the parent pesticidal mode of action	4

On the basis of this investigation there was evidence that a further 67 metabolites could exhibit the parent pesticidal mode of action and an additional 12 will exhibit

pesticidal activity since they work as a pro-pesticide and 3 since they are also a commercial pesticide in their own right. Moreover there is evidence that 237 will not exhibit the pesticidal activity (including the 184 that have had the toxicophore disrupted during degradation). No information was identified to determine whether 166 metabolites would exhibit the pesticidal activity of their parental pesticide.

3.3 Metabolite selection for further study

A number of metabolites were identified as potentially exhibiting their parental pesticide mode of action (see section 3.2) or exhibiting alternative mammalian toxicological endpoints estimated by the approaches DEREK and TOPKAT (see section 3.1). From these compounds 53 metabolites where selected for further study. Initially the 485 metabolites were prioritised based on their exposure index calculated using the approach of Sinclair et al. (2006). This approach provides a normalized value and considers three components important in determining the extent to which raw sources waters may be exposed to pesticide metabolites: extent of environmental formation, mobility and persistence.

Experimentally derived data for mobility (K_{oc}) (31% of metabolites) and soil persistence (DT_{50}) (21% of metabolites) was used in preference. In the absence of experimental data, K_{oc} was estimated and a default value was used for soil DT_{50} (30 days) since no suitable predictive technique has been identified to satisfactorily estimate DT_{50} in any environmental media for pesticide metabolites (Sinclair and Boxall 2005; Sinclair 2009) (For the dataset of metabolites with experimental DT_{50} data, 27 days is the 75th percentile). The Fera PUS supplied annual pesticide usage estimates. Those pesticides without annual usage estimates because they are too 'new' to have been encountered during surveying had their anticipated usage estimated (Miles Thomas per. comm., Fera PUS). Pesticides that lost their market authorisation in the past three years had usage calculated as an average of their usage over the past three years.

All metabolites were ranked according to their calculated exposure index and then the highest ranked metabolites that fulfil a variety of criteria were selected, providing a diverse selection of metabolites with varying concerns for further study. The fifteen highest ranked metabolites that have the potential to exhibit pesticidal activity were selected for further study and these were combined with the ten highest ranked metabolites estimated to exhibit:

- Carcinogenicity by either DEREK or TOPKAT;
- Mutagenicity by either DEREK or TOPKAT;
- Thyroid toxicity (5) or tetratogenicity (5) by DEREK;
- Developmental toxicity by TOPKAT; and
- High rat oral toxicity (<50 mg kg^{-1} body weight) by TOPKAT.

When combined, and due to overlap, this generated a list of 53 metabolites for further study (Appendix 6).

3.3.1 Examination of these compounds as mammalian metabolites

The 53 selected metabolites were investigated to identify whether, as well being a product of the environmental degradation of a pesticide, they are also formed during the mammalian metabolism of the pesticide. Data on mammalian metabolism present in regulatory review documents and the pathway compendiums of Roberts (1998) and Roberts and Hutson (1999) were used for this purpose. 27 of the selected metabolites were identified in mammalian metabolism pathways for the parent pesticides, 24 metabolites were not identified in the available mammalian metabolism pathways and a pathway could not be identified for 2 pesticides each of which had one selected metabolite. An indication of the presence of the selected metabolites in mammalian metabolism pathways is provided in Appendix 6. The detection of a soil metabolite in mammalian metabolism studies is important because it implies that the animals were systemically exposed to the metabolite during the toxicity studies on the parent compound. This gives a degree of reassurance that any adverse effect from the metabolite may have been identified during the studies on the parent compound.

4 Estimation of likely concentrations of metabolites in drinking waters

In England and Wales, drinking water abstracted from surface water gives the largest risk for contamination with metabolites from pesticides. Most surface waters receive discharge directly from agricultural fields through the use of subsurface drainage systems in the fields. Due to the short pathway in comparison with pesticide leaching to groundwater, we can assume that concentrations in surface waters following drainage are much higher than concentrations in groundwater. In England and Wales approximately 68% of the land used for agriculture is drained, which is around 41% of the total land area of England and Wales (SEISMIC v2.0.6; Hollis et al. 1993).

Three surface water abstraction catchments in England were selected following discussion with DWI. Two were selected because the Voluntary Initiative has previously identified them as high-risk catchments for pesticide contamination (Voluntary Initiative 2009) and a further one was selected because the EA has atrazine metabolite monitoring data available. It was decided that during the reporting of this project the catchments would remain anonymous, subsequently identified as Catchment A, B and C.

The methodology for the estimation of metabolite concentrations in surface water was adapted from a study undertaken on behalf of the Pesticide Safety Directorate into the controlling factors for pesticide losses via drainflow (Defra 2008). An empirical relationship was used to predict the concentration in drainflow from the mobility and persistence of the compound and the properties of the soil. This relationship was derived by statistical analysis of a large set of field data collected from literature. The same approach was used here to estimate the concentrations of the metabolites in surface water, using existing knowledge on the mobility and persistence of the compounds, and given the usage of the parent compound and the formation fraction of the metabolite in soil.

The catchment scenarios were characterised by overlaying the information on pesticide use, cropping areas and soil types in a catchment. Only the soil types that are likely to be drained contribute to the discharge of metabolites into the surface water. The maximum concentration in surface water is likely to occur during wet periods from late autumn to early spring. Discharge from drains occurs soon after

rainfall during wet periods when the soil moisture content is already near field capacity. The concentrations in drainflow are usually highest after intense rainfall. Therefore we may assume that the maximum concentration in the surface water will be a cumulative result of all drained fields on which a compound was applied. The concentration is calculated on an area-basis assuming that the drainage water is diluted with metabolite-free solution from other areas, however no allowance for any dilution from spring water inputs are accounted for. Areas contributing metabolite-free water are crop areas where the parent pesticide was not applied, areas with soils that are not expected to be drained and non-cropped areas, e.g. non-agricultural land.

4.1 GIS analysis for the catchments

Areas covered by individual soil series and crop types had to be calculated for each catchment. A spatial analysis was undertaken using the following datasets and methodology.

4.1.1 GIS Datasets

Ward-level agricultural statistics: Agricultural statistics from the June 2003 ward-level agricultural statistics survey (Defra 2003) were used for this study. The statistics provide areas cultivated with a particular crop at the ward level. The data were extracted for all wards that are completely or partly within the catchment boundaries. Agricultural survey data yield an accurate indication of cropped areas in each ward, but do not give any information on the exact location within the ward. The location of the crop areas within the wards was estimated from the Land Cover Map (LCM 2000).

Land Cover Map 2000: Land cover data developed by the Centre for Ecology and Hydrology (CEH 2000) were available at a much higher spatial resolution than the agricultural survey data. The LCM 2000 data set is a classification of broad land cover types for each twenty-five meter square derived from satellite remote sensing. The LCM 2000 land cover classes arable cereals (4.1), arable & horticulture (4.2), arable non-rotational (4.3) and permanent grass (5.1) were used. The crop categories from the agricultural statistics were assigned to one of these four classifications (Table 8). The LCM 2000 data were used to determine the location of agricultural production within each ward and also to discard those sections of a ward that were outside the catchment boundaries.

Table 8. LCM 2000 land cover classes and crop categories within the three catchments that fall within these classes.

LCM 2000 code	Crop categories from agricultural statistics
4.1	Wheat, spring barley, winter barley, oats, maize, other cereals
4.2	Oilseed, linseed, sugarbeet, root crops, brassicas and fodder beet for stock feeding, other food stock, potatoes, field beans and (harvest dry) peas, all other vegetables and salad grown in the open, other arable crops
4.3	Fallow, set-aside

Cropping areas for small fruit and for bulbs and flowers were not available at ward level. These crops are expected to be of minor importance within the three catchments and were not included in the study. Cropping areas for peas and beans and vining peas were also not available at ward level. These areas were accounted for by adjusting the areas of field beans and dry peas by a factor 1.15, based on the total crop areas for peas and beans, vining peas, field beans and dry peas in England in June 2005 (Defra 2007). Orchards, grassland, woodland and non-agricultural areas were also not included in the study. These types of land use are not expected on drained land and do not contribute to surface waters via drainage.

Soil Data: Soil data were available from LandIS (NSRI 2006). The National Soil Map NATMAP vector shows the location of 297 distinct soil associations in England and Wales at the 1:250,000 scale. Soil associations are groups of soil series that occur together in the landscape. A soil series can occur in multiple associations. The NATMAP_ASSOCIATIONS relational table details the soil association to soil series relationships including the expected percentage of each series within the association. Typical organic carbon and clay contents in the top horizon of each soil series were taken from SOILSERIES and HORIZON tabular data. Information on which soil series are likely to be drained within each catchment was available from SSLRC Bulletins (Jarvis et al. 1984, Ragg et al. 1984, Findlay et al. 1984).

Ward Boundaries: Ward boundaries were taken from the 2002 version of the Ordnance Survey Boundary-Line dataset (http://www.ordnancesurvey.co.uk/oswebsite/products/boundaryline/). It contains all levels of electoral and administrative boundaries, from district, wards and civil parishes (or communities) up to parliamentary, assembly and European constituencies at a 1:10,000 scale. The information is represented as vector digital data.

Catchment boundaries: Catchment boundaries were taken from the EA dataset of Water Framework Directive river catchments. This dataset comprises approximately 6,600 catchments in England and Wales, derived by the EA from 50-metre elevation data and corrected to 1:50,000 scale hydrology from the Centre for Ecology and Hydrology.

4.1.2 GIS processing

The methodology that was used to derive areas for each crop/soil combination within a catchment is described in detail in Appendix 7. All spatial processing was performed in ArcDesktop 9.1 using geoprocessing scripts written in PYTHON. A spatial intersect was made of the LCM/soil/ward maps resulting in polygons. There is some uncertainty associated with the identification of land cover class using satellite imagery. Areas per LCM class based on the agricultural survey data are more accurate than those in the LCM 2000 database. The areas within each polygon were therefore adjusted using a correction factor specific to each ward and LCM class. The corrected total area within each LCM/soil/ward polygon was then allocated to individual crops. This was based on ward-specific proportions of individual crops within each LCM class calculated from the agricultural survey data. This cropping/soil dataset for each catchment was then interrogated to determine areas of unique combinations of soil series and crop types in each of the three categories, e.g. the total area of Denchworth soil in Catchment A was divided to calculate the area under wheat, the area under oilseed rape, the area under grass etc.

4.2 Selection of pesticide approvals

The 53 selected metabolites are formed from 38 pesticides. Approvals for these pesticides were extracted from the LIASON database (http://liaison.csl.gov.uk/) for all arable crops for which approvals were listed in the database. The maximum application rate and allowed number of applications during a crop season were collated and the maximum total dose was calculated unless specified in the approval. Each crop was assigned to one of the 19 crop categories mentioned above. The allowed application dose (in kg active ingredient per hectare) was often the same for crops within the same category. The absolute maximum was selected for each crop category from all available approvals. Approvals for which usage had expired by the 1st of January 2009 were not considered. Table 9 shows the selected maximum allowed dose for each pesticide/crop combination. For nine metabolites all approvals

for use of the parent compound had expired by January 2009. Eight metabolites for which use had recently expired (2006-2008) were considered in a separate modelling exercise. The use of metosulam had expired before 2006 and its metabolite was not considered in the modelling.

Pesticide usage varies between areas and years and no actual usage data was available at the catchment scale. A conservative estimate of pesticide usage in the catchments was made based on approvals for use on the crops within the catchment: A pesticide was assumed to be applied on all approved crops at the maximum allowed dose. For example, a pesticide that is approved for use on cereals and potatoes is assumed to be applied at the maximum allowed dose on all cereal and potato crops within the catchment. Repeat applications were treated as a single application at the maximum total dose.

A more refined estimate was made based on survey data on actual pesticide usage in the counties in which the three catchments were situated (Miles Thomas per. comm., Fera PUS). The actual usage in the catchments was uncertain because the counties cover a much larger area than the catchments and the land use and crop cover will vary between different areas of the counties. Nevertheless, a rough estimate of the actual usage could be calculated assuming that usage is evenly distributed within each county. The usage data for each crop (Table 9) was adjusted with a correction factor based on the estimated actual usage in the catchment. Table 10 shows a comparison between the conservative estimate of usage in the catchment based on the maximum allowed application doses for each crop in the catchment, and the estimated actual usage based on the survey data at county level. Often the estimate based on survey data was much smaller than the estimate based on maximum approved usage. However for some pesticides the survey usage was higher. This is most likely due to use on areas that were not considered in the modelling such as non-agricultural land, forest or grassland. These areas were not included in this study because the land is not likely to be drained. Other reasons may be due to pesticides that are only used on minor crops such as flowers and fruits, and due to use on crops that are less represented in the catchment than in the counties.

Table 9. Maximum allowed total pesticide application dosage per crop growth season (kg a.i./ha) for each pesticide and crop

Active ingredient	Fieldbean	Linseed	Maize	O.Arable	Oats	O.Cereal	O.Crops	Oilseed	O.Stock	Potatoes	S.Barley	Setaside	Sugarbeet	Root Stock	Veg&Salad	W.Barley	Wheat
1,3-dichloropropene							423.00			211.50							
Asulam							1.68		2.24						1.20		
Azoxystrobin	0.50			0.50	0.50	0.50	1.00	0.50	0.50	1.50	0.50		0.36	0.50	1.00	0.50	0.50
Carbendazim						0.50		0.75			0.25		0.08			0.25	0.50
Carboxin					0.10	0.10					0.11					0.10	0.11
Chloridazon													3.87	1.82	3.25		
Chlorothalonil	3.02	3.00		3.00	1.00	2.00	1.87	3.02	3.03	7.58	2.00				6.06	2.00	2.00
Chlorotoluron						3.51					3.51					3.51	3.51
Diflufenican					0.13	0.99					0.13					0.99	0.99
Florasulam					0.01	0.01					0.01					0.01	0.01
Fluazifop-P-butyl	0.19	0.19		0.19			0.38	0.38	0.38			0.19	0.38	0.38	0.38		
Flufenacet			0.41			0.24	0.41			0.60					0.41	0.24	0.24
Fosetyl-aluminium							1.33		0.41						4.80		
Glyphosate	1.44	1.76		3.24	2.88		1.80	2.88	1.44		2.88	2.65	0.59	0.59	3.60	2.88	2.88
Imidacloprid		0.10		0.03	0.05	0.06	0.13	0.01					0.09	0.09	1.73	0.06	0.06
Iodosulfuron-methyl						0.01					0.01					0.00	0.01
Ioxynil				0.17	0.48	0.40					0.48				0.63	0.48	0.48
Isoproturon						1.50					1.50					1.50	1.50
Metaldehyde	0.90	0.90	0.90		0.90	0.90	0.90	0.90	0.90	0.35	0.90		0.90	0.90	0.94	0.90	0.90
Metazachlor		1.50		1.50				1.50						0.75	1.50		
Methiocarb			0.44		0.44	0.44	0.22	0.30		0.45	0.44		0.22		0.40	0.44	0.44
Metribuzin										1.40					1.40		
Metsulfuron-methyl		0.01			0.01	0.01	0.01				0.01	0.01				0.01	0.01
Oxamyl										5.50			0.90	0.90	2.00		
Pendimethalin	1.32		1.50			1.32	1.32		1.50	1.32	1.32				1.50	1.32	1.32
Picolinafen																0.05	0.05
Pymetrozine							0.40			0.30					0.40		
Quinmerac		0.25		0.25				0.25					0.39	0.27	0.25		
Tri-allate	2.25			2.25		2.25			2.25		2.25		2.25	2.25	2.25	2.25	2.25
Trifluralin	2.34	1.75			1.20	0.96		2.34					2.34	2.34	2.34	2.50	2.50

Table 10. Comparison between usage based on maximum allowed total pesticide application dosage per crop and estimated usage based on survey data at county level (kg a.i. per catchment area).

Active ingredient	Catchment A		Catchment B		Catchment C	
	Max usage (kg)	Actual usage (kg)	Max usage (kg)	Actual usage (kg)	Max usage (kg)	Actual usage (kg)
1,3-dichloropropene	0	0	6201	0	2573	0
Asulam*	0.08	4.5*	36	0	113	645*
Azoxystrobin	15704	21	8067	6.0	17906	0
Carbendazim	14645	1.7	7349	2.8	14707	4.9
Carboxin	2473	0	1250	0	3297	27
Chloridazon	0	0	36	0	279	0
Chlorothalonil	71668	166	36627	41	77234	105
Chlorotoluron	76620	51	37498	339	99005	0
Diflufenican	21369	13	10622	78	22082	1.5
Florasulam	168	2.4	85	0.3	223	0
Fluazifop-P-butyl	3045	0	1477	0	2886	1.2
Flufenacet	5218	90	2584	96	5650	200
Fosetyl-aluminium*	0.015	0.5*	57	2.9	380	1.0
Glyphosate	89173	948	43987	569	114210	6944
Imidacloprid	1438	77	753	48	1618	108
Iodosulfuron-methyl	208	0	104	0	257	0
Ioxynil	10639	0.8	5389	5.4	14220	6.5
Isoproturon	32745	1669	16025	4300	42338	2052
Metaldehyde	28471	81	14499	63	33328	14
Metazachlor	9287	282	4748	423	7319	29
Methiocarb	11764	129	5944	82	14937	381
Metribuzin	0	0	56	0	127	0
Metsulfuron-methyl	141	4.8	68	0	209	0.2
Oxamyl	0	0	183	0	236	0
Pendimethalin	32862	927	16276	2182	41113	1904
Picolinafen	1127	0	559	0	1115	1.9
Pymetrozine	0	0	13	0	35	0.06
Quinmerac	1548	57	791	16	1222	4.5
Tri-allate	55604	0	27623	0	67426	0
Trifluralin	75548	856	38443	658	68789	1301

*Larger actual usage very likely due to usage on forestry and grassland (asulam) or on minor crops such as fruit and flowers (asulam and fosetyl-aluminium).

4.3 Estimation of metabolite concentrations in drainage water

The concentration of metabolite in drainage water was predicted for each soil-crop combination in the catchment. The maximum concentration in drainflow during a season was predicted from the application dose of pesticide (kg active ingredient per hectare), the formation fraction of the metabolite, the properties of the soil (organic

carbon content and clay content) and the properties of the metabolite, i.e. its soil degradation half-life (DT_{50}) and sorption constant (K_{oc}).

The concentrations were calculated using an empirical relationship that was derived for pesticides in Defra project PS2218 (Design of a targeted mitigation system for transport of pesticides in drainflow in the UK). The statistical relationship was based on 167 records of literature data from 21 European field studies where concentrations in the drains were monitored. Four factors were identified as important influences on the maximum concentration of pesticide in drainage effluent. These were: 1, the time interval between when the pesticide was applied and the occurrence of the first subsequent drainage event (*Interval*); 2, strength of sorption of the pesticide to soil (*Kd*); 3, the clay content of the soil (*Clay%*); and 4, the degradation half-life of the pesticide in soil (DT_{50}).

The relationship can be described by:

$$\begin{aligned}\mathrm{Ln}(Maxconc) = {} & -3.15 - 3.62\cdot10^{-2}\,Interval + 4.86\cdot10^{-1}\,\mathrm{Ln}(Kd) + 1.84\cdot10^{-1}\,Clay\% + 1.36\,\mathrm{Ln}(DT_{50}) \\ & - 3.06\cdot10^{-3}\,Interval.\mathrm{Ln}(Kd) - 5.39\cdot10^{-4}\,Interval.clay\% + 6.09\cdot10^{-3}\,Interval.\mathrm{Ln}(DT_{50}) \\ & - 1.51\cdot10^{-2}\,\mathrm{Ln}(Kd).clay\% - 1.53\cdot10^{-1}\,\mathrm{Ln}(Kd).\mathrm{Ln}(DT_{50}) - 3.01\cdot10^{-2}\,clay\%.\mathrm{Ln}(DT^{50})\end{aligned}$$

in which *Maxconc* is the maximum observed concentration, standardised to the equivalent value assuming an application of 1 kg a.i./ha.

The model can be used for metabolites as well as for parent pesticides after some modification. Metabolites will be formed over time after application of the parent compound. The timing and height of the peak concentration will depend on the degradation rates of the parent and metabolite and the formation fraction. The peak concentration in the field was estimated by multiplying the formation fraction reported from laboratory studies with the application dose of the parent pesticide (kg a.i./ha).

For a metabolite, the interval to drainage refers to the time between when the metabolite peak occurs and when the first drainage event takes place. Drainage will occur during rainfall events on wet soil when the water content of the soil exceeds the field capacity. In England, soil wetness can be expected to be close to field capacity during the wet season from late autumn to early spring. Field capacity lasts

on average from the 14th of November until the 15th April in Catchment B and for longer periods in the two other catchments.

For a conservative estimate of the metabolite concentrations in surface waters, the metabolite peaks are assumed to occur during the wet period. The interval between the peak concentration and the onset of drainage was set to 3 days for all metabolites. The degradation half-life in soil (DT_{50}) was not available for all metabolites and therefore the DT_{50} was set to the recommended default value of 300 days (European Commission 2002) and the usage data used was based on maximum allowed total pesticide application dosage per crop within a catchment. The values generated by this approach were termed 'Conservative Estimate'.

Two refinements of these estimates were performed to provide more realistic estimates using refined input parameters: 1, A more realistic DT_{50} of 30 days, based on the degradation half-lives of metabolites for which experimental DT_{50} were available (~75th percentile), rather than the conservative default value (300d) (termed 'Refined Estimate I'); and 2, A more refined estimate was made based on survey data on actual pesticide usage in the counties in which the three catchments were situated rather than the maximum allowed total pesticide application dosage per crop (termed 'Refined Estimate II').

An investigation was also made on whether replacing the conservative estimate of time to drainage (3 days) with real application timings from monthly pesticide usage and hence realistic interval to drainage would affect estimated concentrations. This was found to have very limited impact when compared to the use of real usage data and more realistic DT_{50} values and therefore the results are not reported.

4.4 Estimation of metabolite concentrations in downstream surface water

The timing of the metabolite peak will occur at different times during the year for the different soils and crops, depending on the application timing and the degradation half-lives of the metabolite and parent compound for the different soil types. The conservative approach used here assumes that the metabolite peaks will occur at the same time for all drained soils within the catchment so that the maximum concentration in the drainage water from all the fields will occur simultaneously.

The maximum concentration in drainage water is very likely to occur during a wet period, i.e. after intensive or prolonged rainfall. At this time the soils in the catchment will be wet (moisture content will have reached field capacity) and the rainfall in the catchment will be surplus water. We assume that during this period all areas in the catchment (including naturally drained soils, non-agricultural areas and runoff from upland slopes) will contribute equally to the water flow in the river.

Based on this assumption the concentration in the river will be diluted proportionally to the areas in the catchments. Estimated concentrations for metabolites in downstream surface waters for the three catchments are provided in Appendix 8.

4.5 Estimation of metabolite concentrations in finished drinking waters

Concentrations in downstream surface waters were then adjusted to allow for the estimated extent removal by drinking water treatment processes (DWTP). There have been a number of recent studies that have looked at the concentration of pesticide metabolites in the environment, source waters and produced drinking waters (Parsons et al. 2006). Hadlick et al. (2008) recently reported the results from a survey of 12 drinking waters in the midwestern United States where they looked for degradates of a number of chloracetamide and triazine pesticides. 19 of the 20 degradates they looked for were found in tap water during a peak (spring) sampling survey although at levels well below any regulatory limits in the USA. The median concentrations of the two atrazine metabolites, i.e. DEA and DIA, were 19 and 8 ng/L respectively (maximum concentrations for all metabolites quantified in this study are provided in Appendix 6). Maximum levels of 320 ng/L for DEA and 75 ng/L for DIA were measured which would be more of a concern in the EU where regulations for individual pesticides and its relevant metabolites must not exceed 0.1 µg/L (100 ng/L), with a total pesticide concentration not exceeding 0.5 µg/L (EU 1998). A number of chloracetamide metabolites were also found at levels above the 0.1 µg/L standard including metalochlor ethanesulfonic acid which was found at a maximum concentration of 1500 ng/L. As there is currently little or no published data on pesticide metabolite removal by DWTPs, removal by specific treatment processes has been estimated here based on the physico-chemical properties of the selected metabolites.

The DWTPs investigated are listed below with a description of each process (Parsons and Jefferson, 2006) and subsequent sections describe how removal was calculated.

1. Coagulation is the process of adding chemical reagents (iron or aluminium salts) in a mixing tank to destabilise colloidal particles and allow them to agglomerate or flocculate with other suspended particles to form larger, more readily settled particles.

2. Activated carbon is a broad-scale adsorbent of dissolved substances. Dissolved, colloidal and particulate substances are attracted and attached to the surface of the carbon particles. It is used to remove taste and odour causing compounds as well as toxic organic chemicals. Precipitation and other chemical reactions also occur on the carbon surface. A variety of carbon adsorbers can be designed, including batch and continuous flow units. The adsorption capacity of the carbon is eventually exhausted. The carbon is regenerated by heating the carbon, which burns and volatilises the substances accumulated on it. The activated carbon can take the form of granules (granular activated carbon – GAC) or powder (powdered activated carbon – PAC).

3. Ozone (O_3) is a more powerful oxidising agent than chlorine and a very effective biocide. Ozone reacts with most organic matter by attacking it directly or through the formation of hydroxyl radicals (•OH) formed by the depletion of ozone.

4. Chlorine (Cl_2) is by far the most common oxidant used in water treatment. Primarily used as a disinfectant but also for iron and manganese removal its efficacy is pH and dose dependent.

In addition to determining removal by specific WTPs, two treatment scenarios were considered. The first was ‘conventional treatment’ consisting of coagulation-flocculation/filtration, powdered activated carbon and chlorination. The second is ‘advanced treatment’ consisting of coagulation-flocculation/filtration, powdered activated carbon, ozonation and chlorination.

4.5.1 Removal via coagulation-flocculation/filtration

In general coagulation-flocculation/filtration will only remove charged colloidal species from water (Parsons and Jefferson 2006). Species that are negatively charged (anionic) at pH 7 are more amenable to removal. We would expect no removal of small uncharged molecules during coagulation and limited removal (25%) of small charged molecules. Any metabolites sorbed to particulate matter are likely to be removed during these processes.

4.5.2 Removal via powdered activated carbon

We have also included powdered activated carbon in the conventional and advanced treatment schemes as it is unlikely that a surface water treatment works with a known pesticide problem would have no barrier to removal. Each pesticide metabolite was put into one of the following categories based on the octanol/water coefficient (K_{ow}) and the charge of compound at pH 7 (Drewes et al. 2007):

1. log K_{ow} > 4 (pH 7); uncharged
2. log K_{ow} = 0–4 (pH 7); uncharged
3. log K_{ow} < 0 (pH 7); uncharged
4. log K_{ow} = 0–1.5 (pH 7); protonated base
5. log K_{ow} < 0 (pH 7); protonated base
6. log K_{ow} = 0–2.5 (pH 7); deprotonated acid
7. log K_{ow} < 0 (pH 7); deprotonated acid

The removal for each category was as follows:

1. >90% removal
2. 90-50% removal
3. 50-25% removal
4. 90-50% removal
5. 50-25% removal
6. 50-25% removal
7. <25% removal

4.5.3 Removal via chlorination and ozonation

We utilised quantitative structure-property relationship (QSPR) models developed and validated by Lei and Snyder (2007) to predict percent reactivity with ozone and free chlorine (HOCl/OCl-) for the selected metabolites. The method is described below.

4.5.3.1 2D to 3D conversion

The mol files of the 53 metabolites selected for study were converted from two-dimensional (2D) representations to three-dimensional (3D) ones, using LigPrep

software (via Maestro). LigPrep uses an energy minimizational approach to calculating 3D structures from 2D structures. Assumptions used for this conversion included an assumed pH of 7 (±2) which sets the ionisation of the metabolite to that most likely in a water treatment scenario. Ionisation of a compound affects the metabolites charge and, hence, reactivity especially with respect to electrophilic reactants such as ozone and chlorine. Additionally, ionisation has a significant effect on other properties of compounds such as sorption to solids (including activated carbon) and volatilisation.

4.5.3.2 Metabolite property estimation

The properties of each metabolite were estimated using QikProp (via Maestro). QikProp makes its property predictions based on the 3D structure calculated using LigPrep. While many molecular properties were estimated, of key interest with respect to the predicted reactivity with ozone and chlorine were: 1) the number of reactive functional groups that were unstable and subject to nucleophilic attack (#rtvFG), 2) the number of likely reaction pathways via electrophilic pathways (#metab), 3) the pi (C-H) component (PISA) of the total solvent accessible surface area (SASA) of the metabolite, 4) the weakly polar component (WPSA) of the SASA, 5) the predicted octanol/water partition coefficient (QP log Kow), and 6) the calculated ionisation potential.

4.5.3.3 Estimation of reactivity

A QSPR approach was used as a screening tool to estimate the reactivity of each of the metabolites. The approach used was based on work by Lei and Snyder (2007). The correlations developed utilized 55 biologically-active compounds as a training set for both ozone and chlorine. The correlations were validated on an independent set of biologically-active compounds. The correlation for percent ozone removal utilised was developed by Lei and Snyder (2007). Specifically, the correlation for percent removal via ozonation was:

$$\% \text{ ozone removal} = 67.3 + 0.0506 \bullet PISA + 5.20 \bullet \#metab - 4.34 \bullet \#rtvFG - 0.114 \bullet WPSA$$

The calculated % ozone removal was used to categorise metabolites as highly, moderately, or slightly reactive with ozone. The independent parameters in the model were PISA, the pi (carbon and attached hydrogen) component of SASA (total solvent accessible surface area) in square angstroms, using a probe with 1.4 angstrom radius); WPSA, the weakly polar component of SASA; #metab, the number

of metabolites amenable to electrophilic attack; and #rtvFG, the number of unstable functional groups suseptible to nucleophilic attack. Each of these parameters was determined using QikProp software (Schrodinger, New York, NY, USA).

In developing the correlation, Lei and Snyder calibrated and validated the model using a set of 62 pharmaceuticals. Of these 62 pharmaceuticals, the QSPR model for % ozone removal was developed and calibrated based on 55 randomly selected pharmaceuticals (of the 62). The model was validated on the remaining compounds. For each calibration compound, QikProp was used to generate 32 descriptors, which were reduced to 25 descriptors by elimination of strictly pharmacological properties. Lei and Snyder found that inclusion of the 21 descriptors from QikProp beyond PISA, #metab, #rtvFG, and WPSA did not dramatically improve the correlation for % ozone removal. Hence, these four descriptors were selected for use in the QSPR model for % ozone removal. Of the four descriptors, WPSA (the π component of the surface area) was by far the most important descriptor, and provided a regression coefficient (R2) of approximately 0.7 by itself. In the QSPR model, addition of the other three selected descriptors brought the regression coefficient to above 0.8.

The QSPR model for percent removal during chlorination (% chlorine removal) was developed by Lei and Synder (2007) in the same manner as the QSPR for % ozone removal. The resulting QSPR was:

$$\%\ \text{chlorine removal} = 106.8 + 0.791 \bullet (\%\ \text{ozone removal}) + 7.89 \bullet \#\text{rtvFG} + 4.80 \bullet \text{QP log Kow} + 0.175 \bullet \text{FISA} - 15.0 \bullet \text{IP}$$

The calculated % chlorine removal was used to categorise metabolites as highly, moderately, or slightly reactive with chlorine. The independent parameters used were FISA (hydrophilic component of SASA, SASA on N, O, and H on heteroatoms; QP log Kow (predicted octanol/water partition coefficient); #rtvFG (# or reactive functional groups); IP (PM3 calculated ionisation potential), and % ozone reactivity (calculated as described above).

4.5.3.4 Reactivity to ozone and chlorine

The predicted percent removals via ozonation (grouped by percent removal) are presented in Appendix 9 Table A9.1, along with the parameters used in the ozone correlation (i.e., #rtvFG, PISA, WPSA, and #metab). Additionally listed is the solvent accessible surface area (SASA) for each compound. For easy comparison, the

corresponding percent removals via chlorination are provided for each compound. The predicted percent removals via chlorination (grouped by percent removal) are presented in Appendix 9 Table A9.2, along with the parameters used in the chlorine correlation, i.e. #rtvFG, FISA, QP log Kow, and IP. For easy comparison, the corresponding percent removals via ozonation are provided for each compound.

Of the 53 metabolites, computational errors did not allow prediction of properties via QikProp for four compounds: 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid, 3-carbamyl-2,4,5-trichlorobenzoic acid, phosphorous acid and acetaldehyde. The reason for the computation errors is not completely clear. Thus, predictions were made for percent removals for 49 metabolites.

For ozonation, the results showed that of the 49 metabolites, 10 were “highly reactive” (summing the 80-89% and 90-100% removal groups), 30 were “moderately reactive” (summing the 70-79% and 60-69% removal groups), and 10 had “low reactivity” (the 10 at 50-59% removal groups) (Appendix 9 Table A9.1). For chlorination, there was generally lower reactivity. Specifically, for chlorination, the results showed that, of the 49 metabolites, only 3 were “highly reactive” (only corresponding to the 80-89% group, with none at the 90-100% removal rate), 16 were “moderately reactive” (summing the 5 at 70-79% and 11 at 60-69%); and 30 had “low reactivity” (summing 15 at 50-59% , 9 at 40-49%, 5 at 30-39%, and 1 at 0-9%) (Appendix 9 Table A9.2).

In percentages, for ozonation, 20% of the compounds were “highly” reactive (80-100% removal), 61% were “moderately” reactive (60-79% removal), and 20% were “poorly” reactive (<60% removal). In percentages, for chlorination, only 6% of the compounds were “highly” reactive (80-100% removal), 33% were “moderately” reactive (60-79% removal), and 61% were “poorly” reactive (<60% removal).

A histogram of the percent removals is presented in Figure 4.1 that again shows the larger number of removals predicted for ozonation versus chlorination. Percent removal via ozonation versus chlorination was significantly correlated ($\alpha<0.000$, $r=0.773$, ref. SPSS v. 17.0) (Figure 4.2). Ozone provides greater removal than chlorine for all except two metabolites, ATSA and cyanazine acid. Cyanazine acid was relatively recalcitrant to oxidation by either ozone or chlorine, while ATSA was relatively more labile.

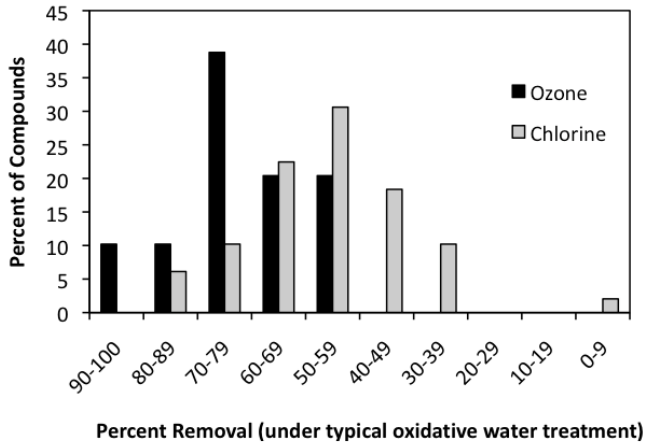

Figure 4.1. Frequency distribution for percent removal under typical oxidative water treatment conditions via ozone and free chlorine (HOCl/OCl-)

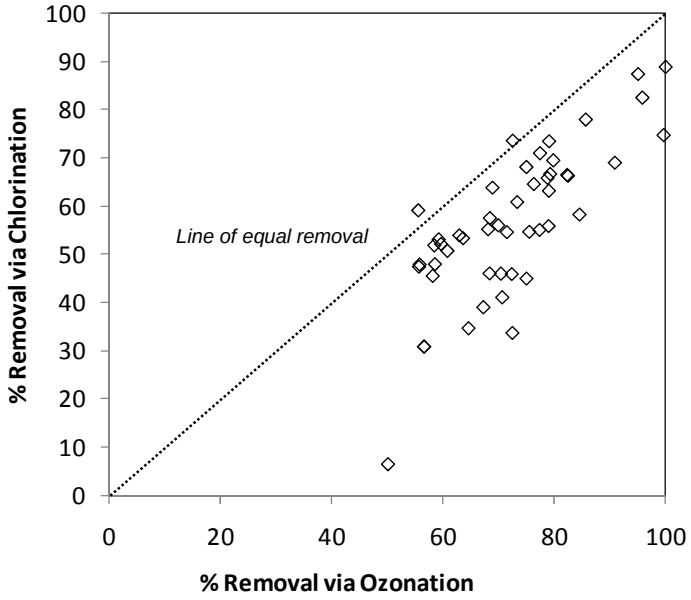

Figure 4.2. Correlation between percent removal via ozonation versus chlorination (Correlation was significant at p<0.000)

4.5.4 Conventional treatment

We have included powdered activated carbon (PAC) in conventional treatment as it is unlikely for a DWTPs with a historical pesticide problem not to have some level of treatment. The predicted removals of each selected metabolite are shown below for coagulation-flocculation, powdered activated carbon and chlorination, together with total estimated removal for the scheme (Table 11). Filtration is not expected to give any further removal as the remaining pesticide metabolites would be fully dissolved.

Table 11. Pesticide metabolite removal by conventional treatment

	Removal percentage with			
Pesticide metabolite	**coagulation**	**PAC**	**chlorination**	**All**
cis-3-chloroprop-2-enoic acid	25	35	30.8	66.3
trans-3-chloroprop-2-enoic acid	25	35	30.8	66.3
aldicarb sulfone	0	15	39	48.2
aldicarb sulfoxide	0	15	46	54.1
sulfanilamide	0	15	69.5	74.1
deethylatrazine	25	50	47.7	80.4
reference compound 10	25	50	73.4	90.0
2-aminobenzimidazole	0	50	70.9	85.5
carbofuran	0	75	55.9	89.0
carboxin sulfoxide	0	35	69	79.9
5-amino-4-chloropyridazin-3(2H)-one	0	15	60.8	66.7
3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	25	35	- [a]	51.3
3-carbamyl-2,4,5-trichlorobenzoic acid	25	50	- [a]	62.5
3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	25	50	50.7	81.5
R417888	0	15	52.1	59.3
3-(3-chloro-p-tolyl)-1-methylurea	0	75	54.6	88.7
cyanazine acid	0	75	59.1	89.8
AE 0542291	0	75	45.9	86.5
5-hydroxy-XDE-570	25	50	66.4	87.4
5-trifluoromethyl-pryid-2-one	0	50	34.7	67.4
FOE oxalte	25	50	53.1	82.4
thiadone	0	50	6.5	53.3
ethanol	0	15	55.2	61.9
phosphorous acid	0	15	- [a]	15.0
aminomethylphosphonic acid	25	15	55.1	71.4
1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	0	15	54.7	61.5
AE F145740	25	75	68.1	94.0
metsulfuron-methyl	25	50	66.3	87.4
3,5-di-iodo-4-hydroxybenzamide	25	50	53.3	82.5
3,5-di-iodo-4-hydroxybenzoic acid	25	90	51.8	96.4
3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	0	50	65.8	82.9
desmethylisoproturon	0	75	63.2	90.8
acetaldehyde	0	15	- [a]	15.0
metazachlor oxalic acid	25	35	77.9	89.2

Pesticide metabolite	Removal percentage with coagulation	PAC	chlorination	All
metazachlor sulfonic acid	0	35	74.7	83.6
methiocarb sulfoxide	0	50	58.2	79.1
ATSA	25	75	73.6	95.1
demethyl metoxuron	0	35	56	71.4
diketo metribuzin	0	35	53.9	70.0
IN-D5119	25	35	45	73.2
IN-D5803	25	35	41.1	71.3
dimethyloxamic acid	25	15	47.9	66.8
2,6-dinitro-3,4-xylidine	0	75	82.5	95.6
4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	0	90	88.8	98.9
4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid	25	90	87.4	99.1
4-fluoroaniline	25	35	64.6	82.7
CGA 294849	0	15	57.5	63.9
BH518-2	25	75	63.8	93.2
BH518-4	25	50	66.6	87.5
deisopropylatrazine	0	50	47.9	74.0
methomyl	0	35	45.5	64.6
diisopropylamine	0	50	33.7	66.9
2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	25	90	46	96.0

[a] – it was not possible to calculate removal for these metabolites in these processes

4.5.5 Advanced treatment

Ozone is routinely used to oxidise pesticides in water treatment before removal by activated carbon (Parsons and Jefferson 2006). Ozone reacts with organic compounds either through the direct reaction with molecular ozone or through the formation of free radicals including the hydroxyl radical. Molecular ozone is a selective electrophile that reacts quickly with double bonds, activated aromatic systems and nonprotonated amines. The predicted removals of each selected metabolite are shown below for coagulation-flocculation, ozonation, powdered activated carbon and chlorination, together with total estimated removal for the scheme (Table 12).

Table 12. Pesticide metabolite removal by advanced treatment

Pesticide metabolite	Removal percentage with coagulation	ozonation	PAC	chlorination	All
cis-3-chloroprop-2-enoic acid	25	55.7	35	30.8	85.1
trans-3-chloroprop-2-enoic acid	25	55.7	35	30.8	85.1
aldicarb sulfone	0	67	15	39	82.9
aldicarb sulfoxide	0	70.5	15	46	86.5

	Removal percentage with				
Pesticide metabolite	**coagulation**	**ozonation**	**PAC**	**chlorination**	**All**
sulfanilamide	0	79.8	15	69.5	94.8
deethylatrazine	25	55.8	50	47.7	91.3
reference compound 10	25	79	50	73.4	97.9
2-aminobenzimidazole	0	77.5	50	70.9	96.7
carbofuran	0	79	75	55.9	97.7
carboxin sulfoxide	0	90	35	69	98.0
5-amino-4-chloropyridazin-3(2H)-one	0	73.4	15	60.8	91.1
3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	25	- [a]	35	- [a]	51.3
3-carbamyl-2,4,5-trichlorobenzoic acid	25	- [a]	50	- [a]	62.5
3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	25	60.9	50	50.7	92.8
R417888	0	59.7	15	52.1	83.6
3-(3-chloro-p-tolyl)-1-methylurea	0	78.8	75	54.6	97.6
cyanazine acid	0	55.6	75	59.1	95.5
AE 0542291	0	72.4	75	45.9	96.3
5-hydroxy-XDE-570	25	82.3	50	66.4	97.8
5-trifluoromethyl-pryid-2-one	0	64.6	50	34.7	88.4
FOE oxalte	25	59	50	53.1	92.8
thiadone	0	50	50	6.5	76.6
ethanol	0	68.2	15	55.2	87.9
phosphorous acid	0	- [a]	15	- [a]	15.0
aminomethylphosphonic acid	25	77.4	15	55.1	93.5
1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	0	75.5	15	54.7	90.6
AE F145740	25	75	75	68.1	98.5
metsulfuron-methyl	25	82.5	50	66.3	97.8
3,5-di-iodo-4-hydroxybenzamide	25	63.7	50	53.3	93.6
3,5-di-iodo-4-hydroxybenzoic acid	25	58.5	90	51.8	98.5
3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	0	78.8	50	65.8	96.4
desmethylisoproturon	0	79	75	63.2	98.1
acetaldehyde	0	- [a]	15	- [a]	15.0
metazachlor oxalic acid	25	86	35	77.9	98.5
metazachlor sulfonic acid	0	99	35	74.7	99.8
methiocarb sulfoxide	0	84.5	50	58.2	96.8
ATSA	25	72.6	75	73.6	98.6
demethyl metoxuron	0	70	35	56	91.4
diketo metribuzin	0	63	35	53.9	88.9
IN-D5119	25	75	35	45	93.3
IN-D5803	25	70.7	35	41.1	91.6
dimethyloxamic acid	25	58.6	15	47.9	86.2
2,6-dinitro-3,4-xylidine	0	95.8	75	82.5	99.8
4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	0	100	90	88.8	100.0
4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid	25	95	90	87.4	100.0
4-fluoroaniline	25	76.4	35	64.6	95.9
CGA 294849	0	68.5	15	57.5	88.6
BH518-2	25	68.9	75	63.8	97.9
BH518-4	25	79.3	50	66.6	97.4
deisopropylatrazine	0	55.9	50	47.9	88.5
methomyl	0	58.2	35	45.5	85.2
diisopropylamine	0	72.5	50	33.7	90.9
2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	25	68.4	90	46	98.7

[a] – it was not possible to calculate removal for these metabolites in these processes

These percentage removals due to conventional and advanced treatments were then applied to the estimated concentrations in surface waters for the three catchments using the Conservative Estimate and the Refined Estimate II. These concentrations in finished drinking water were then used for the evaluation of risk to the UK population in the subsequent sections (Appendix 10).

4.6 Concentrations of pesticidally active metabolites

Fifteen of the metabolites selected for further study were selected on the basis that they may exhibit the same pesticidal activity as their parent pesticide. For these compounds it is not the risk posed to consumers that is critical but rather, since they are pesticidal-acting then the Guidance advises that they should be below the 0.1 µg/L for pesticides required by the regulations. Table 14 provides data on the estimated concentrations of these specific compounds in influent and treated waters following conventional and advanced treatments for the three catchments.

Catchment A – Using the conservative estimate, eight metabolites were above the 0.1 µg/L in the influent water and 5 and 3 metabolites were above this value for treated water following conventional and advanced treatment respectively. All concentrations of metabolites were below 0.1 µg/L using the Refined Estimate II for influent water and hence treated water following conventional and advanced treatment.

Catchment B - Using the conservative estimate, fourteen metabolites were above the 0.1 µg/L in the influent water and 9 and 5 metabolites were above this value for treated water following conventional and advanced treatment respectively. When the Refined Estimate II was considered five metabolites were above the 0.1 µg/L in the influent water and 1 metabolite above this value in treated water following conventional and advanced treatment.

Catchment C – Using the conservative estimate, five metabolites were above the 0.1 µg/L in the influent water and none above this value in treated water following conventional and advanced treatment. All concentrations of metabolites were below 0.1 µg/L following the Refined Estimate II for influent and treated waters.

Catchment C was selected because the EA had available surface water monitoring data for the atrazine metabolites deethyatrazine (DEA) and deisopropylatrazine (DIA) (2003). It is probable that these data will not be representative of raw water abstracted for drinking water purposes because:

- The monitoring points are where the river is relatively small and is in all probability a distance from public abstraction points; and
- Parent pesticide usage, particularly of atrazine will be significantly different for the figures used in the modeling, (2003 versus 2007) since atrazine lost UK approval in 2007.

However when the data are compared (Table 13) it can be seen that for DIA the conservative estimated concentration in influent water is larger than the monitored concentration whilst the situation is reversed for DEA.

Table 13. Comparison of monitoring and modelling data for the metabolites DIA and DEA

Concentration (µg/L)	EA maximum observed in Catchment C	Conservative Estimate influent water	Refined Estimate II influent water
deisopropylatrazine	0.149	0.382	<0.001
deethylatrazine	0.0548	<0.001	0.003

It is clear that concentrations of metabolites are higher in Catchment B than the other two. This is not surprising as this catchment was selected because it has previously been identified as a high-risk catchment for pesticide contamination. In this catchment some metabolites of isoproturon, fosetyl-aluminium, atrazine and chlorotoluron are estimated to be present in raw waters at concentrations greater than 0.1 µg/L. Atrazine lost UK approval in 2007 and isoproturon lost approval in June 2009.

Only one metabolite was identified at concentrations of greater than 0.1 µg/L in finished drinking waters when considering the Refined Estimate II (phosphorous acid, highlighted in bold in Table 14). This metabolite had a low estimated removal rate for GAC (15%) and the calculation of removal estimates for chlorination and ozonation were not possible, therefore zero removal during chlorination or ozonation was

assumed, consequently the estimated influent and finished drinking water concentrations are similar (0.23 µg/L and 0.195 µg/L respectively).

The Conclusion Regarding the Peer Review of the Pesticide Risk Assessment of the Active Substance Fosetyl (EFSA 2005), summarises that phosphorous acid:

- exhibits medium to high persistence in soil;
- exhibits low mobility in soil;
- has a minimal potential for groundwater contamination; and
- in surface water would be expected to partition to the sediment and subsequently transform to phosphate.

The EFSA aquatic exposure assessments only consider the spray drift route of entry to surface water, not runoff and/or drainage as considered during this study.

Table 14. Concentrations in influent and following conventional and advanced treatment for metabolites deemed potential pesticidally active

Metabolite	Parent pesticide	Conservative Estimate (µg/L)			Refined Estimate II (µg/L)		
		Inf.[a]	Conv.[b]	Adv.[c]	Inf.[a]	Conv.[b]	Adv.[c]
Catchment A							
aldicarb sulfone	aldicarb	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
aldicarb sulfoxide	aldicarb	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
deethylatrazine	atrazine	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
carboxin sulfoxide	carboxin	1.606	0.324	0.032	<0.001	<0.001	<0.001
3-(3-chloro-p-tolyl)-1-methylurea	chlorotoluron	6.523	0.740	0.157	0.004	<0.001	<0.001
cyanazine acid	cyanazine	16.547	1.692	0.751	0.004	<0.001	<0.001
5-hydroxy-XDE-570	florasulam	0.089	0.011	0.002	0.001	<0.001	<0.001
phosphorous acid	fosetyl-aluminium	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
metsulfuron-methyl	iodosulfuron-methyl	0.108	0.014	0.002	<0.001	<0.001	<0.001
3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	isoproturon	0.522	0.089	0.019	0.027	0.005	0.001
desmethylisoproturon	isoproturon	1.109	0.102	0.021	0.057	0.005	0.001
diketo metribuzin	metribuzin	0.077	0.023	0.009	0.003	0.001	<0.001
BH518-2	quinmerac	0.063	0.004	0.001	0.002	<0.001	<0.001
BH518-4	quinmerac	0.316	0.040	0.008	<0.001	<0.001	<0.001
deisopropylatrazine	simazine	13.100	3.413	1.505	<0.001	<0.001	<0.001
Catchment B							
aldicarb sulfone	aldicarb	16.008	8.300	2.739	<0.001	<0.001	<0.001
aldicarb sulfoxide	aldicarb	0.702	0.322	0.095	<0.001	<0.001	<0.001
deethylatrazine	atrazine	0.007	0.001	0.001	0.112	0.022	0.010
carboxin sulfoxide	carboxin	3.175	0.640	0.064	<0.001	<0.001	<0.001
3-(3-chloro-p-tolyl)-1-methylurea	chlorotoluron	13.030	1.479	0.314	0.118	0.013	0.003
cyanazine acid	cyanazine	30.702	3.139	1.394	<0.001	<0.001	<0.001
5-hydroxy-XDE-570	florasulam	0.140	0.018	0.003	0.001	<0.001	<0.001
phosphorous acid	fosetyl-aluminium	4.479	3.807	3.807	0.230	**0.195**	**0.195**
metsulfuron-methyl	iodosulfuron-methyl	0.174	0.022	0.004	<0.001	<0.001	<0.001
3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	isoproturon	1.015	0.173	0.037	0.272	0.047	0.010
desmethylisoproturon	isoproturon	1.928	0.177	0.037	0.517	0.048	0.010
diketo metribuzin	metribuzin	0.142	0.042	0.016	<0.001	<0.001	<0.001
BH518-2	quinmerac	0.115	0.008	0.002	0.002	<0.001	<0.001
BH518-4	quinmerac	0.663	0.083	0.017	<0.001	<0.001	<0.001
deisopropylatrazine	simazine	23.135	6.027	2.658	<0.001	<0.001	<0.001
Catchment C							
aldicarb sulfone	aldicarb	0.190	0.098	0.032	<0.001	<0.001	<0.001
aldicarb sulfoxide	aldicarb	0.008	0.004	0.001	<0.001	<0.001	<0.001
deethylatrazine	atrazine	<0.001	<0.001	<0.001	0.003	0.001	<0.001
carboxin sulfoxide	carboxin	0.069	0.014	0.001	0.001	<0.001	<0.001
3-(3-chloro-p-tolyl)-1-methylurea	chlorotoluron	0.318	0.036	0.008	<0.001	<0.001	<0.001
cyanazine acid	cyanazine	0.302	0.031	0.014	<0.001	<0.001	<0.001
5-hydroxy-XDE-570	florasulam	0.002	<0.001	<0.001	<0.001	<0.001	<0.001
phosphorous acid	fosetyl-aluminium	0.115	0.098	0.098	<0.001	<0.001	<0.001
metsulfuron-methyl	iodosulfuron-methyl	0.003	<0.001	<0.001	<0.001	<0.001	<0.001
3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	isoproturon	0.023	0.004	0.001	0.001	<0.001	<0.001
desmethylisoproturon	isoproturon	0.036	0.003	0.001	0.002	<0.001	<0.001
diketo metribuzin	metribuzin	0.004	0.001	<0.001	<0.001	<0.001	<0.001
BH518-2	quinmerac	0.002	<0.001	<0.001	<0.001	<0.001	<0.001
BH518-4	quinmerac	0.005	0.001	<0.001	<0.001	<0.001	<0.001
deisopropylatrazine	simazine	0.382	0.099	0.044	<0.001	<0.001	<0.001

[a] – influent water
[b] – finished water following conventional treatment
[c] – finished water following advanced treatment

4.7 Toxicity retention or abatement of metabolites with chlorine and ozone

The objective of this portion of the project was to use the reactivity of selected study metabolites (determined in section 4.5.3), and to predict whether their toxicity will likely be retained or abated upon oxidation. Specifically, the purpose was to understand the likelihood that a metabolite entering a water treatment plant will be oxidised, or not, under typical disinfection conditions with ozone and/or chlorine. For the reactive compounds, oxidation degradates will be formed and are likely to be present at some level in the finished water unless removed via sorption, e.g. to activated carbon, or transformation, e.g. on biologically active filters, in the water treatment plant. It was necessary, therefore, to tentatively identify which metabolites are the oxidative and thus have the potential for their water treatment degradates to either retain the toxic moiety or lose their toxicity through a chemical change in the toxic moiety.

It was originally considered that prediction of the reactive sites for ozone and chlorine could be used to predict specific compounds formed following reactions with ozone and/or chlorine. This could hypothetically be followed by property and toxicity estimation. However, the results clearly demonstrated that many possible reaction pathways (and relative reaction rates) were possible, and that predicting which compounds were likely to form and increase concentrations by using computational means was too uncertain. Making such predictions is risky, at best, and could easily lead to the incorrect expectation that a specific compound might be formed, or conversely, that some other compound water treatment degradate might not be formed. In either case, the results could be misleading. Instead, the computational results of this research should be used to help formulate experimental laboratory (or pilot/full scale) research aimed at identifying and quantifying likely water treatment degradates of pesticide metabolites.

4.7.1 Reactive Site Prediction

Each molecule was examined for likely moieties to be most subject to electrophilic attack by ozone and chlorine. In general, the following concepts where used in the predictions. Electrophilic attack by ozone preferentially occurs at:

1) double bonds;
2) non-protonated amines; and
3) activated aromatic rings.

Activating substituents on aromatic rings often include –OH, -OR (e.g., -O-CH3, etc.), -NH2, and others. Deactivating substituents on aromatic rings often include –COOH, -COR, -NCO, halogens (e.g., -F, -Cl, -Br, -I, -IO2, -CF3, -CCl3, etc.), and some sulfur-based moieties (e.g., -SCH3, -SH, -SCN, -SOCH3, -SO3H, -SO3-, etc.).

Reaction with chlorine is somewhat more complex. Typical reactions for chlorine may include reaction with:

1) amines and amides, e.g. aliphatic amines to chloramines (RR'NH to RR'NCl); aliphatic amides (RCONRR') (similar to amines but less reactive); amino acids (RCH(COOH)(NH2)); monocyclic aromatic hydrocarbons to electrophilic substitution at ortho- or para-position (as influenced by electrons donating or withdrawing substituents), polycyclic aromatic hydrocarbons to C-OH, C=O, C-Cl);
2) chlorination of heterocyclic structures is very hard to predict;
3) reduced sulphur moieties, e.g. thiols or mercaptans (RSH) to disulphide (RSSR) and sulphonic acid (RSO3H); thioether (RDR') to sulphoxide (RSOR'); and S-triazine to sulphoxide (RSOR');
4) activated aromatic rings (as above);
5) unsaturated (double) bonds, e.g. RC=CR' to RCOHCClR';
6) oxygenated moieties, e.g., alcohols, aldehydes, ketones, acids; and
7) other structures.

Knowledge of which chemical moieties express or cause the toxicity related to a given metabolite, juxtaposed against the likely reaction site for chlorine or ozone, allowed an estimate of whether or not an oxidised metabolite is likely to be detoxified or, conversely, to retain is toxicity.

The next section examines the likely moiety(ies) undergoing electrophilic attack by ozone or chlorine, and how this attack corresponds to the likely moiety(ies) causing toxicity. This allows grouping of the metabolites into groups that: 1) are more or less likely to be oxidised in water treatment by ozone or chlorine; and 2) might be detoxified during oxidation versus those that might be expected to retain their toxicity.

4.7.2 Electrophilic reaction sites versus toxic moieties

Each metabolite structure was examined with respect to the likely point of electrophilic attack by ozone or chlorine. Additionally, the project team developed predictions as to which moieties on the metabolites could be expected to impart the toxicological property of each metabolite. For some metabolites, moieties responsible for toxicity (or the point of likely electrophilic attack) are not known.

Table 15 summarizes whether likely ozone and/or chlorine oxidation sites may be known, and whether likely toxic moieties may be known. Based on intersection of knowledge of reactive versus toxic moieties, metabolites are grouped as to whether their oxidation degradates were estimated to be detoxified or whether they were likely to retain toxicity. Appendix 14 provides the structures of all 53 metabolites and details the position of the toxic moiety and the site of probable electrophilic oxidation.

For 32 compounds, both the likely electrophilic reaction site(s) and the toxic moiety were known. For 12 of these compounds, the reaction site and toxic moiety were the same and, hence, the metabolites might be expected to be detoxified via ozonation or chlorination (Table 15). For an additional 9 of these metabolites, some of the toxic moieties were targeted for oxidation, leading to possible detoxification, though with lower likelihood than for the first 12 (Table 15). For 11 other metabolites, the toxic moeity(ies) was(were) not targeted for likely electrophilic oxidation and, hence, detoxification was less likely (especially for the first oxidation byproducts). For the remaining 21 metabolites, the likely site of oxidation and the site of toxicity (or both) were unknown. Hence, for these 21 compounds, no predictions of detoxification could be made.

As discussed above, many reactions are possible, including: 1) chlorination of amines and amides, reduced sulphur moieties, and activated aromatic rings, and 2) ozonation of unsaturated bonds, dealkylation reactions, activated aromatic ring substitutions, and polymerization products. Ring cleavage is also possible in many cases, but typically not as first oxidation byproducts. In general, chlorine substitution will tend to deactivate further electrophilic reactions, e.g. of an aromatic ring (creating a more stable byproduct or degradate). Conversely, hydroxylation will tend to activate aromatic rings and related structures with respect to electrophilic oxidation, making them more labile and less stable.

This portion of the desk study helped group metabolites into highly, moderately and slightly reactive groups for both ozone and chlorine. Ozone was generally more reactive than chlorine. Each metabolite's structure was analysed. Many possibilities for points of attack by ozone and/or chlorine were observed. Due to differences in reaction rates, one or another reaction pathway can be followed leading to widely different predictions of metabolites. For this reason, it was decided not to predict actual degradates of metabolites.

This work also points to which compounds might be more likely to be detoxified versus retaining toxicity upon initial oxidation. Laboratory experiments are required to confirm the predictions made in this desk study. A comprehensive literature search confirmed the lack of published studies on the ozonation or chlorination of most pesticide metabolites, with the notable exception of metabolites of atrazine, simazine, cyanazine, and a few other pesticides (e.g. Adams and Randtke 1992; Hulsey et al. 1993).

Table 15. Summary of whether toxic moieties will be targetered by ozone or chlorine

Metabolite		% removal via [c]		Reaction site known	Toxic moiety known	Moieties targeted by O3 or FC?
Name	Code	Chlorine	Ozone			
Metabolites possibly detoxified by ozone or chlorine oxidation						
cis-3-chloroprop-2-enoic acid	5	57	31	Yes	Yes	All [a]
trans-3-chloroprop-2-enoic acid	6	57	31	Yes	Yes	All [a]
cyanazine acid	9	56	59	Yes	Yes	All [a]
aldicarb sulfone	12	67	39	Yes	Yes	All [a]
2-aminobenzimidazole	18	77	71	Yes	Yes	All [a]
acetaldehyde	21	ND	ND	Yes	Yes	All [a]
carboxin sulfoxide	27	91	69	Yes	Yes	All [a]
desisopropylatrazine	32	56	48	Yes	Yes	All [a]
desethylatrazine	33	56	47	Yes	Yes	All [a]
metsulfuron-methyl	37	82	66	Yes	Yes	All [a]
sulfanilamide	44	80	69	Yes	Yes	All [a]
ATSA	52	73	74	Yes	Yes	All [a]
Metabolites possibly detoxified by ozone or chlorine oxidation (less likelihood)						
desmethylisoproturon	15	79	63	Yes	Yes	Some
2;6-dinitro-3;4-xylidine	16	96	82	Yes	Yes	Some
3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	19	79	66	Yes	Yes	Some
3-(3-chloro-p-tolyl)-1-methylurea	20	72	55	Yes	Yes	Some
aldicarbsulfoxide	24	70	46	Yes	Yes	Some
diketometribuzin	34	63	54	Yes	Yes	Some
5-hydroxy-XDE-570	35	82	66	Yes	Yes	Some
CGA 294849	46	68	57	Yes	Yes	Some
IN-D5803	47	71	41	Yes	Yes	Some
Metabolites unlikely to be detoxified by ozone of chlorine oxidation						
R417888	1	60	52	Yes	Yes	None [b]
3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	3	ND	ND	Yes	Yes	None [b]
3-carbamyl-2,4,5-trichlorobenzoic acid	4	ND	ND	Yes	Yes	None [b]
reference compound 10	22	79	73	Yes	Yes	None [b]
4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	26	100	89	Yes	Yes	None [b]
1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	29	76	55	Yes	Yes	None [b]
3-cyano-6-hydroxy-2;4;5-trichlorobenzamide	30	61	51	Yes	Yes	None [b]
4-[(1-ethylpropyl)amino]-3;5-dinitro-o-toluic acid	31	95	87	Yes	Yes	None [b]
2-ethyl-7-nitro-5(trifluoromethyl) benzimidazole	45	68	46	Yes	Yes	None [b]
AE F145740	50	75	68	Yes	Yes	None [b]
3,5-di-iodo-4hydroxybenzamide	51	64	53	Yes	Yes	None [b]
Metabolites with unknown detoxification due to lack of information on reactive sites or toxic moieties						
metazachlor sulfonic acid	2	100	75	Yes	No	Unknown
FOEoxalate	7	59	53	Yes	No	Unknown
metazachlor oxalic acid	8	86	78	Yes	No	Unknown
phosphorous acid	10	35	155	No	Yes	Unknown
dimethyloxamic acid	11	59	48	No	No	Unknown
AE 0542291	13	72	46	Yes	No	Unknown
thiadone	14	50	6	Yes	No	Unknown
diisopropylamine	17	73	34	No	No	Unknown
5-amino-4-chloro-3-(2H)-pyridazinone	23	73	61	Yes	No	Unknown
BH518-2	25	69	64	No	Yes	Unknown
4-fluoroaniline	28	76	65	Yes	No	Unknown
BH518-4	36	79	67	No	Yes	Unknown
5-trifluoromethyl-pryid2-one	38	65	35	Yes	No	Unknown
IN-D5119	39	75	45	Yes	Yes	Unknown
demethyl metoxuron	40	70	56	Yes	No	Unknown
methiocarb sulfoxide	41	85	58	Yes	No	Unknown
ethanol	42	68	55	Yes	Yes	Unknown
methomyl	43	58	46	Yes	No	Unknown
carbofuran	48	79	56	Yes	No	Unknown
aminomethylphosphon ic acid	49	77	55	Yes	No	Unknown
3,5-di-iodo-4hydroxybenzoic acid	53	59	52	No	Yes	Unknown

[a] - Because all toxic moieties may be targeted by oxidants, oxidized degradates may be non-toxic or less toxic than metabolite.
[b] - Because no toxic moieties may be targeted by oxidants, oxidized degradates may be as toxic as metabolite.
[c] - ND = not determined

5 Determination of risks to human health

An assessment is presented here of the potential risks posed to the general population from indirect exposure to each of the 53 selected pesticide metabolites. The risk assessment for each pesticide metabolite is made taking into account:

- estimated daily intakes of the pesticide metabolite through consumption of drinking water;
- an evaluation of the proportion of the acceptable daily intake (ADI) for the pesticide metabolite that the estimated daily intake represents for the general adult population and for toddlers; and
- consideration of the potential toxicological hazard of the pesticide metabolite.

5.1 Characterisation of Consumer Intake

The theoretical 'reasonable worst-case' indirect exposure of consumers to pesticide metabolites though consumption of drinking water, resulting from the use of the 39 selected, priority pesticides, was estimated. Exposures were based on the Conservative Estimate and Refined Estimate II concentrations of these compounds in drinking water. Data from three catchment areas were considered (Catchments A, B and C) with two types of water treatment applied, namely, Conventional and Advanced.

5.1.1 Intake from drinking water

Potential indirect exposures to the selected metabolites arising from the consumption of drinking water were calculated by combining the estimated concentrations of the compounds present in drinking water with estimates of water consumed per day by adults and children.

The concentrations of pesticide metabolites present in finished drinking waters were estimated as described in Section 4. Concentrations were estimated on the catchment scale and these raw water concentrations were then refined for removal during drinking water treatment.

Daily water ingestion by an adult was assumed to be 2 L; an 'oral correction factor' of 0.66 was applied to this value to derive an estimated daily water ingestion of 1.3L for a toddler (child aged 1–2 years, in this instance; Environment Agency 2009). Calculations of intakes of pesticide metabolites per person, on a bodyweight basis

(µg/kg bw/day), were based on typical bodyweights of 70 kg for an adult and 10.2 kg for a child aged 1–2 years (Environment Agency 2009). The intake of each individual pesticide metabolite from drinking water was expressed as a proportion of the acceptable daily intake (ADI) for both adults and toddlers.

Concentrations of the selected pesticide metabolites in finished drinking water are summarised in Appendix 10. In order to ensure generation of an adequately conservative scenario, it was assumed that all of the drinking water consumed would contain metabolites at the estimated levels on a long-term basis, although it is acknowledged that there will be seasonal variation based on pesticide usage.

The calculated intakes of pesticide metabolites from indirect exposure through drinking water consumption for each catchment area, and comparisons of these with ADIs, are presented in Table 16, Table 17, Table 18 and Appendix 11. These results were used to inform the risk assessments in the next section.

Table 16. Calculated 'worst-case' intake of pesticide metabolites from drinking water for the general adult population and toddlers (as percentage of ADI) for Catchment A

Pesticide Metabolite [a]	Percentage of ADI			
	Adult		Toddler	
	Based on Conservative Estimate [b]	Based on Refined Estimate II [b]	Based on Conservative Estimate [b]	Based on Refined Estimate II [b]
reference compound 10	<0.01	<0.01	<0.01 – 0.01	<0.01
2-aminobenzimidazole	0.04 – 0.16	<0.01	0.16 – 0.71	<0.01
carboxin sulfoxide	0.06 – 0.58	<0.01	0.26 – 2.62	<0.01
3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	1.33 – 1.33	<0.01	6.00 – 6.00	0.01 – 0.01
3-carbamyl-2,4,5-trichlorobenzoic acid	0.01 – 0.01	<0.01	0.06 – 0.06	<0.01
3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	0.03 – 0.08	<0.01	0.15 – 0.38	<0.01
R417888	0.23 – 0.57	<0.01	1.04 – 2.59	<0.01 – 0.01
3-(3-chloro-p-tolyl)-1-methyl-urea	0.01 – 0.05	<0.01	0.05 – 0.24	<0.01
cyanazine acid	1.07 – 2.42	<0.01	**4.86 – 10.95** [c]	<0.01
AE 0542291	<0.01	<0.01	0.01 – 0.02	<0.01
5-trifluoromethyl-pryid-2-one	0.06 – 0.18	<0.01	0.29 – 0.81	<0.01
FOE oxalte	0.09 – 0.21	<0.01	0.40 – 0.97	0.01 – 0.02
thiadone	0.08 – 0.17	<0.01	0.37 – 0.75	0.01 – 0.01
aminomethylphosphonic acid	<0.01	<0.01	<0.01 – 0.01	<0.01
1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	<0.01	<0.01	<0.01 – 0.02	<0.01
3,5-di-iodo-4-hydroxyben-zamide	<0.01	<0.01	<0.01 – 0.01	<0.01
3,5-di-iodo-4-hydroxybenzoic acid	<0.01	<0.01	<0.01 – 0.01	<0.01
3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	<0.01 – 0.02	<0.01	0.02 – 0.08	<0.01
desmethylisoproturon	<0.01 – 0.02	<0.01	0.02 – 0.09	<0.01
acetaldehyde	0.59 – 0.59	<0.01	2.65 – 2.65	0.01 – 0.01
metazachlor oxalic acid	<0.01 – 0.02	<0.01	0.01 – 0.07	<0.01
metazachlor sulfonic acid	<0.01 – 0.06	<0.01	<0.01 – 0.27	<0.01 – 0.01
methiocarb sulfoxide	0.02 – 0.15	<0.01	0.11 – 0.70	<0.01 – 0.01
diketo metribuzin	<0.01 – 0.01	<0.01	0.01 – 0.02	<0.01
dimethyloxamic acid	0.40 – 0.96	0.01 – 0.03	1.81 – 4.36	0.05 – 0.12
CGA 294849	<0.01 – 0.01	<0.01	0.01 – 0.04	<0.01
BH518-4	<0.01	<0.01	<0.01 – 0.01	<0.01
deisopropylatrazine	0.86 – 1.95	<0.01	3.90 – 8.83	<0.01
methomyl	3.57 – 8.53	<0.01	**16.16 – 38.66** [c]	<0.01
diisopropylamine	<0.01 – 0.01	<0.01	0.01 – 0.05	<0.01
2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	<0.01	<0.01	0.01 – 0.02	<0.01

[a] – Only those pesticide metabolites with estimated intake values >0.01 for either adult or toddlers are shown

[b] – Range given reflects two values predicted following Conventional and Advanced treatments

[c] – Reasonable 'worst-case' daily intake from drinking water exceeds 10% of ADI for toddlers or adults in bold

Table 17. Calculated 'worst-case' intake of pesticide metabolites from drinking water for the general adult population and toddlers (as percentage of ADI) for Catchment B

Pesticide Metabolite [a]	Percentage of ADI			
	Adult		Toddler	
	Based on Conservative Estimate [b]	Based on Refined Estimate II [b]	Based on Conservative Estimate [b]	Based on Refined Estimate II [b]
cis-3-chloroprop-2-enoic acid	0.57 – 1.30	<0.01	2.60 – 5.87	<0.01
trans-3-chloroprop-2-enoic acid	0.57 – 1.30	<0.01	2.60 – 5.87	<0.01
aldicarb sulfone	**7.83 – 23.71** [c]	<0.01 - <0.01	**35.45 – 107.41** [c]	<0.01
aldicarb sulfoxide	0.22 – 0.74	<0.01	0.98 – 3.34	<0.01
reference compound 10	<0.01 – 0.01	<0.01	0.01 – 0.03	<0.01
2-aminobenzimidazole	0.07 – 0.30	<0.01	0.30 – 1.34	<0.01
carbofuran	<0.01 – 0.01	<0.01	0.01 – 0.03	<0.01
carboxin sulfoxide	0.11 – 1.14	<0.01	0.52 – 5.17	<0.01
3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	2.62 – 2.62	<0.01	**11.86 – 11.86** [c]	0.01 – 0.01
3-carbamyl-2,4,5-trichlorobenzoic acid	0.03 – 0.03	<0.01	0.12 – 0.12	<0.01
3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	0.07 – 0.17	<0.01	0.30 – 0.77	<0.01
R417888	0.42 – 1.03	<0.01	1.88 – 4.68	<0.01
3-(3-chloro-p-tolyl)-1-methyl-urea	0.02 – 0.11	<0.01	0.10 – 0.48	<0.01
cyanazine acid	1.99 – 4.48	<0.01	**9.02 – 20.31** [c]	<0.01
AE 0542291	<0.01 – 0.01	<0.01	0.01 – 0.04	<0.01
5-trifluoromethyl-pryid-2-one	0.11 – 0.30	<0.01	0.48 – 1.37	<0.01
FOE oxalte	0.17 – 0.41	<0.01 –<0.01	0.76 – 1.86	<0.01
thiadone	0.16 – 0.32	0.01 –0.01	0.73 – 1.46	<0.01
ethanol	<0.01	<0.01	<0.01 – 0.01	<0.01
phosphorous acid	<0.01	<0.01	0.01 – 0.01	<0.01
aminomethylphosphonic acid	<0.01	<0.01	<0.01 – 0.01	<0.01
1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	<0.01 – 0.01	<0.01	0.01 – 0.04	<0.01
3,5-di-iodo-4-hydroxyben-zamide	<0.01	<0.01	0.01 – 0.02	<0.01
3,5-di-iodo-4-hydroxybenzoic acid	<0.01	<0.01	<0.01 – 0.01	<0.01
3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	0.01 – 0.03	<0.01	0.03 – 0.15	0.01 – 0.04
desmethylisoproturon	0.01 – 0.03	<0.01 – 0.01	0.03 – 0.15	0.01 – 0.04
acetaldehyde	1.11 – 1.11	<0.01	5.05 – 5.05	0.02 – 0.02
metazachlor oxalic acid	<0.01 – 0.03	<0.01	0.02 – 0.13	<0.01 – 0.01
metazachlor sulfonic acid	<0.01 – 0.11	<0.01	<0.01 – 0.48	<0.01 – 0.04
methiocarb sulfoxide	0.05 – 0.30	<0.01	0.21 – 1.38	<0.01 – 0.02
ATSA	<0.01	<0.01	<0.01 – 0.01	<0.01
diketo metribuzin	<0.01 – 0.01	<0.01	0.02 – 0.04	<0.01
IN-D5803	<0.01 – 0.01	<0.01	0.01 – 0.03	<0.01
dimethyloxamic acid	0.80 – 1.94	<0.01	3.63 – 8.77	0.49 – 1.18
CGA 294849	<0.01 – 0.01	<0.01	0.02 – 0.06	<0.01
BH518-4	<0.01 -<0.01	<0.01	<0.01 – 0.01	<0.01
deisopropylatrazine	1.52 – 3.44	<0.01	**6.88 – 15.60** [c]	<0.01
methomyl	**7.00 – 16.75** [c]	<0.01	**31.71 – 75.86** [cca]	<0.01 - <0.01
diisopropylamine	0.01 – 0.02	<0.01 - <0.01	0.01 – 0.03	<0.01 - <0.01
2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	<0.01 - <0.01	<0.01 - <0.01	0.01 – 0.02	<0.01 - <0.01

[a] - Only those pesticide metabolites with estimated intake values >0.01 for either adult or toddlers are shown

[b] - Range given reflects two values predicted following Conventional and Advanced treatments

[c] - Reasonable 'worst-case' daily intake from drinking water exceeds 10% of ADI for toddlers or adults in bold

Table 18. Calculated 'worst-case' intake of pesticide metabolites from drinking water for the general adult population and toddlers (as percentage of ADI) for Catchment C

Pesticide Metabolite [a]	Percentage of ADI			
	Adult		Toddler	
	Based on Conservative Estimate [b]	Based on Refined Estimate II [b]	Based on Conservative Estimate [b]	Based on Refined Estimate II [b]
cis-3-chloroprop-2-enoic acid	<0.01	<0.01	<0.01 – 0.01	<0.01
trans-3-chloroprop-2-enoic acid	<0.01	<0.01	<0.01 – 0.01	<0.01
aldicarb sulfone	0.09 – 0.28	<0.01	0.42 – 1.27	<0.01
aldicarb sulfoxide	<0.01 – 0.01	<0.01	0.01 – 0.04	<0.01
2-aminobenzimidazole	<0.01 – 0.01	<0.01	0.01 – 0.02	<0.01
carboxin sulfoxide	<0.01 – 0.03	<0.01	0.01 – 0.11	<0.01
3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	0.05 – 0.05	<0.01	0.22 – 0.22	<0.01
3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	<0.01	<0.01	0.01 – 0.01	<0.01
R417888	0.01 – 0.02	<0.01	0.03 – 0.07	<0.01
3-(3-chloro-p-tolyl)-1-methyl-urea	<0.01	<0.01	<0.01 – 0.01	<0.01
cyanazine acid	0.02 – 0.04	<0.01	0.09 – 0.20	<0.01
5-trifluoromethyl-pryid-2-one	<0.01 – 0.01	<0.01	0.01 – 0.03	<0.01
FOE oxalte	<0.01 – 0.01	<0.01	0.01 – 0.04	<0.01
thiadone	<0.01 – 0.01	<0.01	0.01 – 0.03	<0.01
acetaldehyde	0.02 – 0.02	<0.01	0.09 – 0.09	<0.01
metazachlor sulfonic acid	<0.01	<0.01	<0.01 – 0.01	<0.01
methiocarb sulfoxide	<0.01 – 0.01	<0.01	<0.01 – 0.03	<0.01
dimethyloxamic acid	0.02 – 0.05	<0.01	0.09 – 0.21	<0.01 – 0.01
deisopropylatrazine	0.03 – 0.06	<0.01	0.11 – 0.26	<0.01
methomyl	0.15 – 0.36	<0.01	0.67 – 1.61	<0.01
2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	<0.01	<0.01	0.01 – 0.02	<0.01

[a] - Only those pesticide metabolites with estimated intake values >0.01 for either adult or toddlers are shown

[b] - Range given reflects two values predicted following Conventional and Advanced treatments

[c] - Reasonable 'worst-case' daily intake from drinking water exceeds 10% of ADI for toddlers or adults in bold

5.2 Risk Assessment

5.2.1 Estimated Daily Intake as Proportion of ADI

For each pesticide metabolite, the predicted 'reasonable worst-case' daily intake values for the average consumer (adults and toddlers) were compared with the ADI. The ADI was sourced for each pesticide and, where possible, each pesticide metabolite during preparation of toxicological profiles (Section 5.1.3). For many of the pesticide metabolites, it was not possible to source an ADI and, in such cases, a Project Specific Derived Value (PSDV) was estimated; where used, justification for the level of estimated PSDV is given in the associated toxicological profile (Appendix 12).

The estimated daily intakes and percentage contribution of these intakes to ADIs (where >0.01 for either adults or toddlers) are detailed in Appendix 11. Where the total predicted daily intake from drinking water amounted to <10% of the ADI, it was considered that there was no appreciable risk associated with these sources (Section 5.2.3). Where a potential for exceedence of 10% of ADI was estimated for either adults or toddlers, a more detailed assessment of the extent and nature of the risk was undertaken (Section 5.2.4). A cut-off level of ≥10% was adopted to acknowledge the potential contribution of other dietary sources to the total daily intake of each pesticide metabolite.

5.2.2 Assessment of Toxicological Hazard

In making the risk assessment for those pesticide metabolites where estimated daily intake exceeded 10% of the ADI, the estimated exposure was considered in the light of the nature of the toxicological hazard. Toxicological profiles were prepared for all parent pesticides and metabolites and are detailed in metabolite specific hazard assessments, given in Appendix 12.

Toxicological profiles were based on a critical review of the most current evaluations published by authoritative organisations, such as the World Health Organization/Food and Agriculture Organization (WHO/FAO) Joint Expert Committee on Food Additives (JECFA), Joint Meeting on Pesticide Residues (JMPR), Hazardous Substances Data Bank (HSDB), United States Environmental Protection Agency (US EPA); Pesticide Safety Directorate (PSD), European Commission (EU), Pesticide Properties Database (PPDB) and International Agency for Research on Cancer (IARC). In those many cases where these above authoritative sources did

not provide a complete dataset for the pesticide metabolites, extensive literature searches were further conducted to identify any other relevant published papers. The literature search strategy adopted is described in Appendix 13.

5.2.3 Pesticide metabolites for which estimated worst-case intake for adults and/or toddlers are of no concern

For the selected pesticide metabolites for which the total predicted daily intake from drinking water amounted to less than 10% of the ADI, there was not considered to be any appreciable risk to consumers (adults and toddlers) from this source. Thus, no further detailed consideration was undertaken for the following pesticide metabolites: cis-3-chloroprop-2-enoic acid; trans-3-chloroprop-2-enoic acid; aldicarb sulfoxide; sulphanilamide; deethylatrazine; reference compound 10; 2-aminobenzimidazole; carbofuran; carboxin sulfoxide; 5-amino-4-chloropyridazin-3(2H)-one; 3-carbamyl-2,4,5-trichlorobenzoic acid; 3-cyano-6-hydroxy-2,4,5-trichlorobenzamide; R417888; 3-(3-chloro-p-tolyl)-1-methylurea; AE 0542291; 5-hydroxy-XDE-570; 5-trifluoromethyl-pryid-2-one; FOE oxalte; thiadone; ethanol; phosphorous acid; aminomethylphosphonic acid; 1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine; AE F145740; metsulfuron-methyl; 3,5-di-iodo-4-hydroxybenzamide; 3,5-di-iodo-4-hydroxybenzoic acid; 3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea; desmethylisoproturon; acetaldehyde; metazachlor oxalic acid; metazachlor sulfonic acid; methiocarb sulfoxide; ATSA; demethyl metoxuron; diketo metribuzin; IN-D5119; IN-D5803; dimethyloxamic acid; 2,6-dinitro-3,4-xylidine; 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol; 4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid; 4-fluoroaniline; CGA 294849; BH518-2; BH518-4; diisopropylamine; 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole.

5.2.4 Pesticide metabolites for which the estimated worst-case intake for adults and/or toddlers exceeds 10% of the ADI

Where the predicted daily intake was above 10% of the ADI for either adults or toddlers, but did not exceed the ADI for any of the populations considered, the margin of safety was considered to be still clearly adequate for the source of exposure considered, but this was with the proviso that the individual did not receive other intakes of that pesticide metabolite on a daily basis. In such cases, this might take daily intake above the ADI. Therefore, the nature of the chemical's hazard profile was taken into account in the risk characterisation and to add additional

reassurance to the assessment of the risk level. Assessments of the risks posed by the individual pesticide metabolites are presented below.

5.2.4.1 Aldicarb sulfone

The ADI derived by the NRA (2001) is 1 µg/kg bw/day; this is based on the no-observed-effect level (NOAEL)[1] for inhibition of brain cholinesterase in beagle dogs and includes a safety factor of 100. This was considered appropriate as depressed cholinesterase activity, which was the principal endpoint of concern, has been shown in humans following acute exposure to the parent pesticide aldicarb (Appendix 12.12).

Intake of aldicarb sulfone from drinking water was highlighted as being above the 10% cut-off level for both adults and toddlers in Catchment B. The Conservative Estimate of drinking water levels were predicted to account for 23.71% (0.24 µg/kg bw/day) and 107.41% of the ADI (1.07 ug/kg bw/day), for adults and toddlers respectively, based on conventional water treatment; predicted levels in Catchment B following advanced water treatment were 7.83% (0.08 µg/kg bw/day) and 35.45% (0.35 ug/kg bw/day) of the ADI for adults and toddlers respectively.

However, intake of aldicarb sulfone from drinking water using the Refined Estimate II of drinking water levels did not exceed 10% of the ADI for adults or toddlers, in any of the catchment areas considered.

Hence, the exposure levels from the sources considered herein would not be expected to pose a significant risk taking into account both the exposure, and the nature of the critical toxic effect of aldicarb sulfone.

5.2.4.2 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid

Due to the lack of available human or experimental data, it has not been possible to establish a robust NOAEL for 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid. In addition, no ADI has been proposed for this metabolite by any authoritative organisation. Therefore, for the purposes of this study a ‘project specific derived value’ (PSDV) was derived for use in the risk assessment (Appendix 12.3).

[1] Throughout the document, the terms no-observed-effect level (NOEL) and no-observed-adverse-effect level (NOAEL) have been used as per the source reference.

IPCS (1996) have established an ADI of 18 µg/kg bw/d for the parent chlorothalonil, based on the NOAEL for incidence of papillomas and carcinomas of the forestomach in rats and mice, and a safety factor of 100. The predicted toxicity profile for 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid using TOPKAT and DEREK software is less than that of the parent; applying the same ADI as that of the parent will give a considerable overall margin of safety (>10,000). The lesions, including tumours, seen in the rodent studies are most likely related to sustained irritancy and cytotoxicity and not due to genotoxic effects (IARC, 1999).

Intake of 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid from drinking water was highlighted as being above the 10% cut-off level for toddlers in Catchment B; adult levels did not exceed the cut-off level. The Conservative Estimate of drinking water levels were predicted to account for 11.86% (2.13 µg/kg bw/day) of the ADI for toddlers, based on both conventional and advanced water treatments.

However, intake of 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid from drinking water using the Refined Estimate II of drinking water levels, did not exceed 10% of the ADI for adults or toddlers, in any of the catchment areas considered.

Hence, the exposure levels from the sources considered herein would not be expected to pose a significant risk taking into account both the exposure, and the nature of the critical toxic effect of 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid.

5.2.4.3 Cyanazine chloroacid

Due to the lack of available human or experimental data, it has not been possible to establish a robust NOAEL for cyanazine chloroacid. In addition, no ADI has been proposed for this metabolite by any authoritative organisation. Therefore, for the purposes of this study a ‘project specific derived value’ (PSDV) was derived for use in the risk assessment (Appendix 12.9).

IPCS have established an ADI of 2 µg/kg bw/d for the parent cyanazine, based on the NOAEL for CNS effects, and a safety factor of 100. However, due to the predicted toxicity profile for cyanazine chloroacid using TOPKAT and DEREK software being less than that of the parent; applying the same ADI as that of the parent will give a considerable overall margin of safety (> 10,000).

Intake of cyanazine chloroacid from drinking water was highlighted as being above the 10% cut-off level for toddlers in Catchments A and B; adult levels did not exceed the cut-off level in any of the catchments considered. The Conservative Estimate of drinking water levels were predicted to account for 10.95% (0.22 µg/kg bw/day) and 20.31% (0.41 µg/kg bw/day) of the ADI for toddlers in Catchments A and B respectively, based on conventional water treatment; predicted levels following advanced water treatment were 4.86% (0.10 µg/kg bw/day) and 9.02% (0.18 ug/kg bw/day) of the ADI for toddlers in Catchment A and B respectively.

However, intake of cyanazine chloroacid from drinking water using Refined Estimate II of drinking water levels did not exceed 10% of the ADI for adults or toddlers in any of the catchment areas considered.

Hence, the exposure levels from the sources considered herein would not be expected to pose a significant risk taking into account both the exposure, and the nature of the critical toxic effect of cyanazine chloroacid.

5.2.4.4 Deisopropylatrazine

Due to the lack of available human or experimental data, it has not been possible to establish a robust NOAEL for deisopropylatrazine. In addition, no ADI has been proposed for this metabolite by any authoritative organisation. Therefore, for the purposes of this study a PSDV was derived for use in the risk assessment (Appendix 12.32).

An ADI of 5 µg/kg bw/d has been established by the EPA (2005) for the parent simazine, based on a NOAEL for haematological and blood chemistry effects, with a safety factor of 100. The predicted toxicity profile for deisopropylatrazine using TOPKAT and DEREK software is less than that of the parent; applying the same ADI as that of the parent will give a considerable overall margin of safety (>10,000).

Intake of deisopropylatrazine from drinking water was highlighted as being above the 10% cut-off level for toddlers in Catchment B; adult levels did not exceed the cut-off level. The Conservative Estimate of drinking water levels were predicted to account for 15.60% (0.78 µg/kg bw/day) of the ADI for toddlers, based on conventional water treatment; predicted levels following advanced water treatment were 6.88% (0.34 µg/kg bw/day) of the ADI for toddlers in Catchment B.

Intake of deisopropylatrazine from drinking water using the Refined Estimate II of drinking water levels, did not exceed 10% of the ADI for adults or toddlers in any of the catchment areas considered.
Hence, the exposure levels from the sources considered herein would not be expected to pose a significant risk taking into account both the exposure, and the nature of the critical toxic effect of deisopropylatrazine.

5.2.4.5 Methomyl

The ADI set by the EPA (1988) is 2.5 µg/kg bw/day; this is based on the no-observed-effect level (NOEL) of acetylcholinesterase inhibition and haematological changes in beagle dogs and includes a safety factor of 100. This was considered appropriate as depressed cholinesterase activity, which was the principal endpoint of concern, has been shown in humans following acute exposure to methomyl (Appendix 12.43).

Intake of methomyl from drinking water was highlighted as being above the 10% cut-off level for adults in Catchment B and for toddlers in Catchments A and B. The Conservative Estimate of drinking water levels were predicted to account for 16.75% (0.42 µg/kg bw/day) of the ADI for adults in Catchment B, based on conventional water treatment; predicted levels following advanced water treatment were 7.00% (0.10 µg/kg bw/day) of the ADI respectively.

For toddlers, the Conservative Estimate of drinking water levels were predicted to account for 38.66% (0.97 µg/kg bw/day) and 75.86% (1.90 µg/kg bw/day) of the ADI in Catchments A and B respectively, based on conventional water treatment; predicted levels following advanced water treatment were 16.16% (0.40 µg/kg bw/day) and 31.71% (0.79) of the ADI respectively.

Intake of Methomyl from drinking water using the Refined Estimate II of drinking water levels did not exceed 10% of the ADI for adults or toddlers.

Hence, the exposure levels from the sources considered herein would not be expected to pose a significant risk taking into account both the exposure and the nature of the critical toxic effect of for methomyl.

6 Conclusions

Limited monitoring has been performed by the Environment Agency, water companies or research organisations on the occurrence of pesticide metabolites in surface waters and groundwater, raw abstracted water and finished drinking water in the UK. Most of the available data are for metabolites of DDT and heptachlor with no data at all available for some of the high usage pesticides. Data are available for other countries, particularly the US but these data are generally for triazine and chloroacetamide herbicides used in high quantities in the Mid-Western US and these data are probably not applicable to the UK scenario.

485 soil degradation metabolites were identified from pesticides with current and recently lost UK approval and, of those, 53 were selected for further study on the basis of their potential to contaminate source waters (incorporating measures of estimates of pesticide usage, extent of formation, persistence and mobility), estimated toxicology and/or their potential to exhibit pesticidal activity.

The concentrations of the selected metabolites in raw and treated drinking waters were estimated using a conservative approach which was further refined with more realistic estimates of pesticide usage and/or less conservative default value for dissipation in soil. The highest concentration of any metabolite in finished drinking water when applying the realistic refined estimate was 0.265 µg/L for metazachlor sulfonic acid, a metabolite that does not exhibit pesticidal activity. Estimates for one of the metabolites, phosophorous acid, identified as potentially exhibiting pesticidal activity was above the 0.1 µg/L limit for drinking water. It was not possible to determine removal efficiencies of this compound during ozonation and/or chlorination and consequently zero removal was assumed in reaching the estimate. Removal of this compound by ozonation or chlorination may warrant further investigation. All other metabolites with potential pesticidal activity were below the 0.1 µg/L limit.

The effect of chlorination and/or ozonation on metabolites was investigated and it was possible to determine that some metabolites (21) would be detoxified by these processes and others (11) would not. Estimating the identity of potential compounds formed during chlorination and/or ozonation using computational methods, would produce an excessive number of compounds making any analysis of their potential toxicology very difficult.

The results of this risk assessment investigating exposure to pesticide metabolites through consumption of drinking water from three catchment areas were judged to be generally reassuring. For 48 of the selected metabolites, the estimated consumer intakes from drinking water were less than 10% of the ADI for all sections of the population and catchment areas considered. It is concluded, therefore, that these 48 pesticide metabolites do not pose a potential risk to consumer health.

For five pesticide metabolites the estimated 'reasonable worst-case' intake through drinking water (Conservative Estimate with conventional treatment) was found to be above 10% of the ADI for adults and/or toddlers; all of these cases presented in Catchment B and two also presented in Catchment A. The pesticide metabolites falling into this category were, aldicarb sulfone, 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid, cyanazine chloroacid, deisopropylatrazine and methomyl. However, estimated intake of these pesticide metabolites did not exceed 10% of the ADI when using Refined Estimate II of drinking water concentrations.

A cut-off level of 10% of the ADI was adopted in the risk assessment and pesticide metabolites for which intake in drinking water did not exceed 10% were not discussed in detail. While the 10% level is arbitrary, it was considered reasonable as it allowed a considerable margin for possible exposures from other sources without the ADI being exceeded.

Given the estimated intake of 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid for toddlers in Catchment B (above 100% and 30% of ADI for Conservative and Refined estimates) and the IARC (1999) evaluation of the parent chlorothalonil as Group 2B, possibly carcinogenic to humans, consideration should be given to further investigate actual levels of this metabolite in drinking water. However, from a toxicological perspective, this is not considered a critical concern at low levels. The carcinogenicity classification from IARC is based primarily on renal toxicity and on forestomach lesions, including tumours, due to the irritancy of the substance and the high doses used in the studies. It does not appear to be a genotoxic carcinogen where low level exposure might be a concern.

There is potential for erosion of the margin of safety offered by use of the estimated PSDV for 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid, cyanazine chloroacid and

deisopropylatrazine, as PSDV values are based on predicted toxicity values only. However, as a considerable margin of safety (>10,000) has been built into the use of the PSDVs for each of these pesticide metabolites, this should not impact on current risk assessments.

It is not currently possible to assess fully the potential risk to health posed by the predicted exposure of consumers to many of the pesticide metabolites through drinking water, owing to the absence of an ADI and the lack of robust data from which a NOAEL could be derived. Although the toxicity assessment systems DEREK and TOPKAT have allowed an estimated PSDV for use in risk assessment, these are likely to represent 'worst-case' assumptions.

Therefore considering the work undertaken in this study there is no evidence that the current guidance issued by DWI on the requirements to monitor for pesticide metabolites in drinking waters should be changed.

7 Suggestions

- Whilst the outcome of this project was that the selected metabolites pose limited risk to the UK population, this was a purely desk-based exercise and it would be prudent to validate these conclusions with monitoring of raw and/or treated drinking waters for the presence of pesticide metabolites since no data currently exists for the UK for any metabolites formed from approved pesticides with high usage. Such work could focus on metabolites from high use pesticides and/or metabolites identified in this study that need further investigation, e.g. metazachlor sulfonic acid, phosphorous acid and 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid. If this were to be undertaken careful consideration should be given to the temporal and spatial selection of monitoring sites. It would be important to focus on sites that predominantly use surface waters from heavily agricultural areas and the implementation of sampling regimes that include high risk periods such as the first autumn flush.

- To perform lab-based analytical studies, using state-of-the-art analytical techniques to definitively determine the identity of compounds formed during chlorination and ozonation of metabolites (and pesticides). Consideration

should be given to performing these studies on metabolites identified in this study as: a) highly reactive to chlorine or ozone, and b) as not having their toxic chemical moiety likely targeted by the chlorine or ozone attack. This set of compounds could be further refined to: c) focus on degradates of parent pesticides with higher estimated or known toxicity, and d) focus on metabolites of parent compounds with estimated or known significant occurrence in the environment or drinking water sources.

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Appendix 1 – Environment Agency Pesticide Metabolite Concentrations in Surface Water Summary

Metabolite	Parent Pesticide	Environment Agency Region	Period	Total number of analyses	% of analyses below LOD	Limit of Detection (µg/L)	Maximum concentration (µg/L)	Mean concentration (µg/L)	Number of detects above LOD
op-DDE	DDT	Anglian	07/01/2003 – 18/12/2007	1285	100	0.001 - 0.003	-	-	-
		Midlands	03/01/2003 – 28/12/2007	1447	100	0.001 - 0.1	-	-	-
		Northeast	25/09/2006 – 26/11/2008	17	100	0.001 - 0.02	-	-	-
		Northwest	06/01/2003 – 17/12/2007	1531	99.9	0.001 – 0.5	0.01	-	1
		Southern	14/01/2003 – 19/10/2007	169	100	0.001- 0.01	-	-	-
		Southwest	07/01/2003 – 17/12/2007	314	100	0.001 – 0.01	-	-	-
		Thames	-	-	-	-	-	-	-
		Wales	17/03/2003 – 01/04/2004	33	100	0.002	-	-	-
pp-DDE	DDT	Anglian	07/01/2003 – 18/12/2007	2126	99.9	0.001 – 0.01	0.002	0.0013	3
		Midlands	03/01/2003 – 28/12/2007	1641	100	0.001 – 0.1	-	-	-
		Northeast	02/01/2003 – 19/12/2007	3842	99.1	0.0006 – 0.1	0.02	0.0036	35
		Northwest	06/01/2003 – 18/12/2007	1670	99.6	0.001 – 0.5	0.0986	0.0251	7
		Southern	06/01/2003 – 18/12/2007	1439	95.1	0.001 – 0.02	0.036	0.0041	71
		Southwest	07/01/2003 – 18/12/2007	1489	99.9	0.001 – 0.02	0.002	0.0016	2
		Thames	02/01/2003 – 18/12/2007	2438	99.8	0.001 – 0.01	0.005	0.0023	4
		Wales	03/01/2003 – 18/12/2007	2782	99.9	0.0006 – 0.015	0.002	0.0015	3
deethylatrazine	atrazine	Anglian	13/07/2006 – 16/04/2007	2	50	0.02	0.026	-	1
		Midlands	06/06/2006 – 22/11/2007	55	92.7	0.02 – 0.2	0.041	0.031	4
		Northeast	31/10/2006 – 21/03/2007	5	100	0.02 – 20	-	-	-
		Northwest	01/09/2006 – 11/12/2006	7	100	0.02 – 2	-	-	-
		Southern	28/01/2003 – 25/11/2007	44	95.5	0.02 – 0.054	0.0288	0.0284	2
		Southwest	21/01/2003 – 24/03/2004	53	56.6	0.02 – 0.0677	0.0665	0.0381	23
		Thames	15/10/2007 – 17/12/2007	17	100	0.02 – 0.023	-	-	-
		Wales	-	-	-	-	-	-	-
deisopropylatrazine	atrazine	Anglian	13/07/2006 – 16/04/2007	2	100	0.02	-	-	-
		Midlands	06/06/2006 – 22/11/2007	55	96.4	0.02 – 0.2	0.0584	0.0402	2
		Northeast	31/10/2006 – 21/03/2007	5	100	0.02 – 20	-	-	-
		Northwest	01/09/2006 – 11/12/2006	7	100	0.02 – 2	-	-	-
		Southern	28/01/2003 – 25/11/2007	44	68.2	0.02 – 1.85	0.54	0.1587	14
		Southwest	21/01/2003 – 24/03/2004	53	94.3	0.02 – 0.0265	0.149	0.0691	3
		Thames	15/10/2007 – 17/12/2007	17	64.7	0.02 – 0.023	0.056	0.0335	6
		Wales	-	-	-	-	-	-	-

Metabolite	Parent Pesticide	Environment Agency Region	Period	Total number of analyses	% of analyses below LOD	Limit of Detection (µg/L)	Maximum concentration (µg/L)	Mean concentration (µg/L)	Number of detects above LOD
dementon-S-methyl sulphone	dementon-S-methyl	Anglian	-	-	-	-	-	-	-
		Midlands	-	-	-	-	-	-	-
		Northeast	13/09/2007 – 13/12/2007	9	100	0.01	-	-	-
		Northwest	21/12/2004 – 18/12/2007	633	99.8	0.01	1	-	1
		Southern	27/10/2006 – 18/12/2007	25	100	0.01	-	-	-
		Southwest	-	-	-	-	-	-	-
		Thames	19/11/2007 – 29/11/2007	3	100	0.01	-	-	-
		Wales	10/02/2003 – 12/12/2007	27	100	0.01	-	-	-
cis-heptachlor epoxide	heptachlor	Anglian	07/01/2003 – 18/12/2007	1242	100	0.002 - 0.008	-	-	-
		Midlands	16/06/2005 - 28/12/2007	338	100	0.0023 - 0.05	-	-	-
		Northeast	25/09/2006 – 21/11/2007	16	100	0.0023 - 0.02	-	-	-
		Northwest	03/10/2006 – 19/09/2007	10	100	0.0023 - 1.15	-	-	-
		Southern	06/01/2003 – 18/12/2007	791	100	0.002 - 0.005	-	-	-
		Southwest	04/02/2005 – 19/10/2007	4	100	0.0023 - 0.023	-	-	-
		Thames	11/01/2003 – 05/07/2004	5	100	0.002 - 0.003	-	-	-
		Wales	-	-	-	-	-	-	-
trans-heptachlor epoxide	heptachlor	Anglian	07/01/2003 – 18/12/2007	1242	100	0.0025 – 0.008	-	-	-
		Midlands	16/06/2005 – 28/12/2007	358	99.7	0.0025 – 0.005	0.05	-	1
		Northeast	25/09/2006 – 21/11/2007	17	100	0.003 – 0.02	-	-	-
		Northwest	03/10/2006 – 19/09/2007	41	100	0.003 – 1.25	-	-	-
		Southern	06/01/2003 – 18/12/2007	791	100	0.0025 – 0.005	-	-	-
		Southwest	04/02/2005 – 19/10/2007	4	100	0.003 – 0.025	-	-	-
		Thames	11/01/2003 – 05/07/2004	5	100	0.003	-	-	-
		Wales	-	-	-	-	-	-	-
op-TDE	DDT	Anglian	07/01/2003 – 18/12/2007	1288	99.8	0.001 – 0.006	0.002	0.0017	3
		Midlands	03/01/2003 – 28/12/2007	1443	100	0.001 – 0.1	-	-	-
		Northeast	25/09/2006 – 26/11/2007	18	100	0.001 – 0.034	-	-	-
		Northwest	06/01/2003 – 17/12/2007	1533	100	0.001 – 0.5	0.12	0.0264	5
		Southern	14/01/2003 – 19/10/2007	170	98.8	0.001 – 0.01	0.0042	0.0031	2
		Southwest	04/02/2004 – 19/10/2007	4	100	0.001 – 0.01	-	-	-
		Thames	03/01/2003 – 06/12/2007	861	99.9	0.001 – 0.01	0.002	-	1
		Wales	17/03/2003 – 01/04/2004	34	100	0.002	-	-	-

Metabolite	Parent Pesticide	Environment Agency Region	Period	Total number of analyses	% of analyses below LOD	Limit of Detection (µg/L)	Maximum concentration (µg/L)	Mean concentration (µg/L)	Number of detects above LOD
pp-TDE	DDT	Anglian	07/01/2003 – 18/12/2007	2111	99.2	0.001 – 0.02	0.009	0.0035	16
		Midlands	03/01/2003 – 28/12/2007	1643	100	0.001 – 0.1	-	-	-
		Northeast	02/01/2003 – 19/12/2007	3816	99.1	0.0003 – 0.1	0.11	0.0108	34
		Northwest	06/01/2003 – 18/12/2007	1676	99.4	0.001 – 0.5	0.311	0.0342	10
		Southern	06/01/2003 – 18/12/2007	1434	93.2	0.001 – 0.028	0.235	0.01	97
		Southwest	07/01/2003 – 18/12/2007	1492	99.3	0.001 – 0.0272	0.004	0.002	11
		Thames	03/01/2003 – 06/12/2007	2443	99.1	0.001 – 0.01	0.006	0.0023	23
		Wales	21/05/2007 – 10/12/2007	2785	99.9	0.0003 – 0.02	0.005	0.0035	4

Appendix 2 – Environment Agency Pesticide Metabolite Concentrations in Groundwater Summary

Metabolite	Parent Pesticide	Environment Agency Region	Period	Total number of analyses	% of analyses below LOD	Limit of Detection (µg/L)	Maximum concentration (µg/L)	Mean concentration (µg/L)	Number of detects above LOD
op-DDE	DDT	Anglian	02/06/2003 – 18/12/2007	1292	100	0.001 - 0.02	-	-	-
		Midlands	28/01/2003 – 19/12/2007	473	99.8	0.001 - 0.025	0.003	-	1
		Northeast	01/03/2004 – 20/12/2007	365	98.9	0.0001 - 0.005	0.007	0.0055	4
		Northwest	01/12/2003 – 08/08/2007	598	100	0.001 – 0.025	-	-	-
		Southern	10/07/2003 – 29/12/2007	661	100	0.001- 0.01	-	-	-
		Southwest	26/11/2003 – 13/12/2007	339	100	0.001 – 0.004	-	-	-
		Thames	08/09/2004 – 14/12/2007	470	100	0.001- 0.01	-	-	-
		Wales	03/03/2003 – 07/12/2004	176	93.2	0.001 – 0.005	0.01	0.006	12
pp-DDE	DDT	Anglian	02/06/2003 – 18/12/2007	1292	99.2	0.001 – 0.02	0.055	0.0106	10
		Midlands	28/01/2003 – 19/12/2007	929	99.7	0.001 – 0.025	0.008	0.004	3
		Northeast	03/03/2003 – 20/12/2007	665	99.4	0.0006 – 0.01	0.007	0.0043	4
		Northwest	01/12/2003 – 08/08/2007	598	99.8	0.001 – 0.025	0.005	-	1
		Southern	02/01/2003 – 29/12/2007	1609	99.8	0.001 – 0.01	0.016	0.005	4
		Southwest	26/11/2003 – 13/12/2007	339	99.7	0.001 – 0.004	0.001	-	1
		Thames	08/09/2004 – 14/12/2007	470	100	0.001 – 0.01	-	-	-
		Wales	03/01/2003 – 18/12/2007	298	100	0.001 – 0.005	-	-	-
deethylatrazine	atrazine	Anglian	10/03/2004 – 18/12/2007	1533	87.3	0.02 – 0.5	0.923	0.1	195
		Midlands	17/10/2003 – 19/12/2007	1592	91.9	0.02 – 0.107	0.697	0.078	129
		Northeast	01/03/2004 – 20/12/2007	796	95.4	0.004 – 1	0.517	0.09	37
		Northwest	07/06/2006 – 15/11/2007	901	95	0.02 – 0.025	0.281	0.06	45
		Southern	13/10/2003 – 30/10/2007	356	80.9	0.02 – 0.03	0.362	0.062	68
		Southwest	04/12/2003 – 13/12/2007	769	86.0	0.02 – 0.2	0.379	0.057	108
		Thames	23/08/2004 – 14/12/2007	799	82.1	0.02 – 0.06	0.353	0.067	143
		Wales	19/07/2004 – 25/10/2007	577	86.3	0.02 – 0.2	1.02	0.143	79
deisopropylatrazine	atrazine	Anglian	10/03/2004 – 18/12/2007	1541	88.0	0.02 – 0.2	0.812	0.082	185
		Midlands	17/10/2003 – 19/12/2007	1595	95.1	0.02 – 0.107	0.914	0.103	78
		Northeast	01/03/2004 – 20/12/2007	794	96.6	0.003 – 1	0.462	0.076	27
		Northwest	08/06/2004 – 15/11/2007	897	99.0	0.02 – 2	0.206	0.089	9
		Southern	13/10/2003 – 30/10/2007	357	93.0	0.02 – 0.04	0.612	0.113	25
		Southwest	04/12/2003 – 13/12/2007	769	96.6	0.02 – 0.2	0.235	0.072	26
		Thames	23/08/2004 – 14/12/2007	800	91.5	0.02 – 0.182	0.342	0.061	68
		Wales	19/07/2004 – 25/10/2007	579	94.8	0.02 – 0.2	0.449	0.117	30

Metabolite	Parent Pesticide	Environment Agency Region	Period	Total number of analyses	% of analyses below LOD	Limit of Detection (µg/L)	Maximum concentration (µg/L)	Mean concentration (µg/L)	Number of detects above LOD
cis-heptachlor epoxide	heptachlor	Anglian	02/06/2003 – 18/12/2007	1289	100	0.002 - 0.046	-	-	-
		Midlands	03/11/2003 - 19/12/2007	750	99.9	0.001 – 2.5	0.0024	-	1
		Northeast	01/03/2004 – 20/12/2007	367	98.4	0.0003 - 0.012	0.011	0.003	6
		Northwest	07/06/2004 – 08/08/2007	375	100	0.002 – 0.005	-	-	-
		Southern	15/04/2003 – 30/10/2007	392	100	0.002 - 0.01	-	-	-
		Southwest	26/11/2003 – 13/12/2007	343	100	0.002 - 0.009	-	-	-
		Thames	08/09/2004 – 14/12/2007	472	100	0.002 - 0.03	-	-	-
		Wales	23/03/2005 – 07/12/2007	186	100	0.0023 – 0.011	-	-	-
trans-heptachlor epoxide	heptachlor	Anglian	02/06/2003 – 18/12/2007	1278	100	0.0025 – 0.05	-	-	-
		Midlands	03/11/2003 – 19/12/2007	747	99.9	0.001 – 2.7	0.0087	-	1
		Northeast	01/03/2004 – 20/12/2007	361	99.2	0.001 – 0.013	0.0133	0.0054	3
		Northwest	07/06/2004 – 08/08/2007	375	100	0.0025 – 0.005	-	-	-
		Southern	15/04/2003 – 30/10/2007	393	100	0.0025 – 0.01	-	-	-
		Southwest	26/11/2003 – 13/12/2007	338	100	0.0025 – 0.01	-	-	-
		Thames	09/01/2004 – 14/12/2007	549	100	0.0025 – 0.03	-	-	-
		Wales	23/03/2005 – 07/12/2007	183	100	0.0025 – 0.012	-	-	-
op-TDE	DDT	Anglian	02/06/2003 – 18/12/2007	1291	99.6	0.001 – 0.02	0.004	0.0024	5
		Midlands	28/01/2003 – 19/12/2007	930	99.9	0.001 – 0.025	0.003	-	1
		Northeast	03/03/2003 – 20/12/2007	635	98.6	0.0005 – 0.009	0.0185	0.0071	9
		Northwest	01/12/2003 – 08/08/2007	598	99.8	0.001 – 0.025	0.002	-	1
		Southern	28/01/2003 – 06/1012007	228	98.7	0.001 – 0.01	0.02	0.009	3
		Southwest	26/11/2003 – 13/12/2007	339	100	0.001 – 0.004	-	-	-
		Thames	08/09/2004 – 14/12/2007	469	100	0.001 – 0.01	-	-	-
		Wales	22/01/2003 – 07/12/2007	310	96.1	0.001 – 0.005	0.01	0.0063	12
pp-TDE	DDT	Anglian	02/06/2003 – 18/12/2007	1290	99.1	0.001 – 0.02	0.012	0.003	12
		Midlands	28/01/2003 – 19/12/2007	927	99.6	0.001 – 0.025	0.003	0.003	4
		Northeast	03/03/2003 – 20/12/2007	659	98.9	0.0003 – 0.01	0.01	0.0039	7
		Northwest	01/12/2003 – 08/08/2007	596	99.2	0.001 – 0.025	0.004	0.0028	5
		Southern	02/01/2003 – 06/11/2007	1132	99.7	0.001 – 0.01	0.005	0.003	3
		Southwest	26/11/2003 – 13/12/2007	338	98.5	0.001 – 0.004	0.003	0.0018	5
		Thames	08/09/2004 – 14/12/2007	469	98.7	0.001 – 0.01	0.008	0.0037	6
		Wales	03/03/2003 – 07/12/2007	292	100	0.001– 0.005	-	-	-

Appendix 3 – Water Company Pesticide Metabolite Concentrations in Raw, Treated and Consumers Taps Summary

Metabolite	Parent Pesticide [a]	Water type	Period	Total number of analyses	% of analyses below LOD	Limit of Detection (µg/L)	Maximum concentration (µg/L)	Mean concentration (µg/L)	Number of detects above LOD
heptachlor epoxide	**heptachlor**								
	Company 1	Groundwater - raw	03/02/2005 – 14/07/2008	56	100	0.001 – 0.003	-	-	-
		Groundwater – treated	31/01/2005 – 23/06/2008	173	95.4	0.001 – 0.003	0.01	0.0035	8
		Surface water - raw	02/02/2005 – 14/07/2008	27	85.2	0.001 – 0.003	0.003	0.002	4
		Surface water - treated	31/01/2005 – 23/06/2008	31	93.5	0.001 – 0.003	0.001	0.001	2
	Company 2	Treated	04/2008 – 04/2009	28	100	0.01	-	-	-
	Company 3	Groundwater – raw	Post 2004	250	100	0.002	-	-	-
		Groundwater – treated	Post 2004	11	100	0.002	-	-	-
		Surface water – raw	Post 2004	1867	100	0.002			
		Surface water – treated	Post 2004	668	100	0.002			
		Customer taps	Post 2004	6068	100	0.002			
	Company 4	Raw	02/01/2004 – 16/06/2008	677	87	0.007 - 0.01	0.01	0.0072	88
		Customers taps	02/01/2004 – 31/11/2005	2696	85.3	0.007 - 0.01	0.01	0.0071	397
	Company 5	Raw	25/02/2008 – 22/08/2008	216	100	0.002	-	-	-
		Treated	26/02/2008 – 22/08/2008	80	100	0.002	-	-	-
		Customers taps	25/02/2008 – 21/08/2008	187	99.5	0.002	0.004	-	1
pp-DDE	**DDT**								
	Company 2	Treated	04/2008 – 04/2009	28	100	-	-	-	-
	Company 3	Groundwater – raw	Post 2004	237	100	0.002	-	-	-
		Groundwater – treated	Post 2004	8	100	0.002	-	-	-

Metabolite	Parent Pesticide [a]	Water type	Period	Total number of analyses	% of analyses below LOD	Limit of Detection (µg/L)	Maximum concentration (µg/L)	Mean concentration (µg/L)	Number of detects above LOD
		Surface water – raw	Post 2004	1851	100	0.002			
		Surface water – treated	Post 2004	654	100	0.002			
		Customer taps	Post 2004	4639	100	0.002			
	Company 5	Raw	25/02/2008 – 22/08/2008	216	100	0.002	-	-	-
		Treated	26/02/2008 – 22/08/2008	80	100	0.002	-	-	-
		Customers taps	25/02/2008 – 21/08/2008	187	99.5	0.002	0.004	-	1
op-DDE	**DDT**								
	Company 2	Treated	04/2008 – 04/2009	28	100	-	-	-	-
	Company 3	Groundwater – raw	Post 2004	243	100	0.002	-	-	-
		Groundwater – treated	Post 2004	10	100	0.002	-	-	-
		Surface water – raw	Post 2004	1856	100	0.002			
		Surface water – treated	Post 2004	658	100	0.002			
		Customer taps	Post 2004	4639	100	0.002			
	Company 5	Raw	25/02/2008 – 22/08/2008	216	100	0.002	-	-	-
		Treated	26/02/2008 – 22/08/2008	80	100	0.002	-	-	-
		Customers taps	25/02/2008 – 21/08/2008	187	99.5	0.002	0.004	-	1
op-TDE	**DDT**								
	Company 2	Treated	04/2008 – 04/2009	28	100	-	-	-	-
	Company 3	Groundwater – raw	Post 2004	239	100	0.002	-	-	-
		Groundwater – treated	Post 2004	10	100	0.002	-	-	-
		Surface water – raw	Post 2004	1861	100	0.002			

Metabolite	Parent Pesticide [a]	Water type	Period	Total number of analyses	% of analyses below LOD	Limit of Detection (µg/L)	Maximum concentration (µg/L)	Mean concentration (µg/L)	Number of detects above LOD
		Surface water – treated	Post 2004	660	100	0.002			
		Customer taps	Post 2004	4639	100	0.002			
	Company 5	Raw	25/02/2008 – 22/08/2008	216	100	0.002	-	-	-
		Treated	26/02/2008 – 22/08/2008	80	100	0.002	-	-	-
		Customers taps	25/02/2008 – 21/08/2008	187	99.5	0.002	0.004	-	1
pp-TDE	**DDT**								
	Company 2	Treated	04/2008 – 04/2009	28	100	-	-	-	-
	Company 3	Groundwater – raw	Post 2004	239	100	0.003	-	-	-
		Groundwater – treated	Post 2004	10	100	0.003	-	-	-
		Surface water – raw	Post 2004	1855	100	0.003			
		Surface water – treated	Post 2004	656	100	0.003			
		Customer taps	Post 2004	4639	100	0.003			
	Company 5	Raw	25/02/2008 – 22/08/2008	216	100	0.003	-	-	-
		Treated	26/02/2008 – 22/08/2008	80	100	0.003	-	-	-
		Customers taps	25/02/2008 – 21/08/2008	187	99.5	0.003	0.006	-	1
deethylatrazine	**atrazine**								
	Company 6	Groundwater – raw	29/10/2003 – 08/05/2008	401	-	-	0.325	0.0963	401
		Groundwater – treated	29/10/2003 – 13/05/2008	101	-	-	0.099	0.0575	101
	Company 7	Groundwater - raw	01/05/2003 – 31/05/2008	102	1	0.005	0.092	0.0235	101
		Groundwater - treated	01/05/2003 – 19/06/2008	144	69.4	0.005	0.055	0.0234	44

Metabolite	Parent Pesticide [a]	Water type	Period	Total number of analyses	% of analyses below LOD	Limit of Detection (µg/L)	Maximum concentration (µg/L)	Mean concentration (µg/L)	Number of detects above LOD
		Surface water – treated	14/04/2003 – 01/07/2008	154	76.6	0.005	0.007	0.0054	36
		Customers taps	08/04/2003 – 08/07/2008	129	60.5	0.005	0.052	0.0161	51
deisopropylatrazine	**atrazine**								
	Company 7	Groundwater - raw	01/05/2003 – 31/05/2008	102	65.7	0.008	0.024	0.0125	35
		Groundwater - treated	01/05/2003 – 19/06/2008	144	91.7	0.008	0.018	0.0113	12
		Surface water – treated	14/04/2003 – 01/07/2008	154	100	0.008	-	-	-
		Customers taps	08/04/2003 – 08/07/2008	129	93.0	0.008	0.0015	0.0124	9

[a] – At the request of DWI the identity of the water companies who provided data have been made anonymous. Some of the entries are a combined submission for multiple companies

Appendix 4 – Literature Metabolite Concentrations in Surface Waters, Groundwaters and Raw and Treated Drinking Water Summary

Environmental compartment	Transformation product	Parent pesticide [a]	Concentration	Limit of detection	Country	Reference
Vadose zone water						
	alachlor ethane sulfonic acid	alachlor	3 - 73 µg L^{-1}	0.5 µg L^{-1}	USA	(Aga and Thurman, 2001)
	deethylatrazine	atrazine	0.3 µg L^{-1} [c]	0.04 µg L^{-1}	USA	(Steinheimer and Scoggin, 2001)
			9 - 19 µg L^{-1} [b]	0.1 µg L^{-1}	USA	(Fermanich et al., 1996)
			0.76 - 1.48 µg L^{-1} [b]	0.04 µg L^{-1}	USA	(Pashin et al., 2000)
			15 - 29 µg L^{-1} [b]	-	USA	(Mills and Thurman, 1994)
			4.7 - 22.1 µg L^{-1} [b]	0.02 µg L^{-1}	USA	(Adams and Thurman, 1991)
	deisopropylatrazine	atrazine	0.6 µg L^{-1} [c]	0.04 µg L^{-1}	USA	(Steinheimer and Scoggin, 2001)
			< 0.5 µg L^{-1}	0.2 µg L^{-1}	USA	(Fermanich et al., 1996)
			0.11 - 0.78 µg L^{-1}	0.03 µg L^{-1}	USA	(Pashin et al., 2000)
			7 - 15 µg L^{-1} [b]	-	USA	(Mills and Thurman, 1994)
			< 0.02 µg L^{-1}	0.02 µg L^{-1}	USA	(Adams and Thurman, 1991)
	didealkylatrazine	atrazine	0.2 - 1.25 µg L^{-1}	0.03 µg L^{-1}	USA	(Pashin et al., 2000)
	hydroxyatrazine	atrazine	0.08 - 0.37 µg L^{-1}	0.04 µg L^{-1}	USA	(Pashin et al., 2000)
	BH518-2	quinmerac	0.7 µg L^{-1} [b]	0.05 µg L^{-1}	Germany	(PSD, 1998)
	BH518-5	quinmerac	0.16 µg L^{-1} [b]	0.05 µg L^{-1}	Germany	(PSD, 1998)
Leachate						
	2,6-diethylaniline	alachlor	1 µg L^{-1}	-	Italy	(Fava et al., 2000)
	2-chloro-2',6'-diethylacetanilide	alachlor	2.2 - 2.7 µg L^{-1}	-	Italy	(Fava et al., 2000)
	2-hydroxy-2',6'-diethylacetanilide	alachlor	0.8 µg L^{-1}	-	Italy	(Fava et al., 2000)
	aldicarb sulfone	aldicarb	1.5 µg L^{-1}	-	-	(APVMA, 2001)
	aldicarb sulfoxide	aldicarb	0.23 µg L^{-1}	-	-	(APVMA, 2001)
	benalaxyl M1	benalaxyl	4.68 - 4.87 µg L^{-1} [c]	-	Switzerland	(EU, 2004)
	benalaxyl M2	benalaxyl	4.53 - 7.83 µg L^{-1} [c]	-	Switzerland	(EU, 2004)
	2,6-dichlorbenzamide	diclobenil	14.2 - 80.4 ppb	-	Germany	(EPA, 1998c)
	2-ethyl-6-methylaniline	metolachlor	0.6 µg L^{-1}	-	Italy	(Fava et al., 2000)
	trifluoroethanoic acid	flurtamone	2.53 - 11.46 µg L^{-1}	-	UK	(PSD, 2000)
	RE 54488	flurtamone	0.03 - 0.05 µg L^{-1}	-	UK	(PSD, 2000)
	RH-6467	fenbuconazole	trace	-	-	(PSD, 1995)
	RH-9129	fenbuconazole	trace	-	-	(PSD, 1995)
	RH-9130	fenbuconazole	trace	-	-	(PSD, 1995)
	kresoxim-methyl acid	kresoxim-methyl	ND - 0.04 µg L^{-1}	0.01µg L^{-1}	Germany	(PSD, 1997)
Surface water runoff						
	acetochlor oxanilic acid	acetochlor	ND - 0.08 µg L^{-1}	0.01 µg L^{-1}	USA	(Ferrer et al., 1997)
	alachlor ethane sulfonic acid	alachlor	ND - 48.84 µg L^{-1}	0.5 µg L^{-1}	USA	(Aga and Thurman, 2001)

Environmental compartment	Transformation product	Parent pesticide [a]	Concentration	Limit of detection	Country	Reference
runoff	alachlor oxanilic acid	alachlor	ND - 0.17 µg L^{-1}	0.01 µg L^{-1}	USA	(Ferrer et al., 1997)
continued…	deethylatrazine	atrazine	0 - 10.33 µg L^{-1} [d]	-	France	(Patty et al., 1997)
			8 - 29 µg L^{-1} [b]	0.05 µg L^{-1}	USA	(Thurman et al., 1994)
			0.97 µg L^{-1} [c]	0.02 µg L^{-1}	USA	(Blanchard and Donald, 1997)
	deisopropylatrazine	atrazine	0 - 12.14 µg L^{-1} [d]	-	France	(Patty et al., 1997)
	metolachlor ethane sulfonic acid	metolachlor	ND - 1.26 µg L^{-1}	0.5 µg L^{-1}	USA	(Aga and Thurman, 2001)
			0.05 - 0.47 µg L^{-1}	0.01 µg L^{-1}	USA	(Ferrer et al., 1997)
	metolachlor oxanilic acid	metolachlor	ND - 0.29 µg L^{-1}	0.01 µg L^{-1}	USA	(Ferrer et al., 1997)
tile drain						
	deethylatrazine	atrazine	0.36 - 7.71 µg L^{-1}	0.01 µg L^{-1}	Canada	(Muir and Baker, 1976)
	deisopropylatrazine	atrazine	0.01 - 0.78 µg L^{-1}	0.01 µg L^{-1}	Canada	(Muir and Baker, 1976)
	cyanazine amide	cyanazine	< 0.04 - 3.3 µg L^{-1}	0.01 µg L^{-1}	Canada	(Muir and Baker, 1976)
	deisopropylatrazine	cyanazine	0.02 - 0.62 µg L^{-1}	0.01 µg L^{-1}	Canada	(Muir and Baker, 1976)
	deethylatrazine	cyprazine	0.15 - 3.6 µg L^{-1}	0.01 µg L^{-1}	Canada	(Muir and Baker, 1976)
	metolachlor ethane sulfonic acid	metolachlor	5 - > 20 µg L^{-1}	0.2 µg L^{-1}	USA	(Phillips et al., 1999)
	metolachlor oxanilic acid	metolachlor	1 - 10 µg L^{-1}	0.2 µg L^{-1}	USA	(Phillips et al., 2002)
ditch						
	2,4-D methyl ester	2,4-D	ND	<0.19 µg L^{-1}	USA	(Battaglin et al., 2009)
	2-isoprpyl-6-methyl-4-hydroxypyrimidine	diazinon	ND	1 µg L^{-1}	Canada	(Li et al., 2002)
	diazoxon	diazinon	ND	0.03 µg L^{-1}	Canada	(Li et al., 2002)
	deisopropylatrazine	atrazine, cyanazine and simazine	0.17 µg L^{-1}	0.08 µg L^{-1}	USA	(Battaglin et al., 2009)
	hydroxyatrazine	atrazine	0.682 µg L^{-1}	-	USA	(Battaglin et al., 2009)
	diaminochlorotriazine	atrazine	0.062 µg L^{-1}	0.04 µg L^{-1}	USA	(Battaglin et al., 2009)
	aminomethylphosphonic acid	glyphosate	2.9 µg L^{-1}	0.02 µg L^{-1}	USA	(Battaglin et al., 2009)
stream						
	acetochlor ethane sulfonic acid	acetochlor	< 0.2 - 1.6 µg L^{-1}	0.2 µg L^{-1}	USA	(Kalkhoff et al., 2003)
	acetochlor oxanilic acid	acetochlor	< 0.02 - 1.4 µg L^{-1}	0.2 µg L^{-1}	USA	(Kalkhoff et al., 2003)
	2,6-diethylanaline	alachlor	ND	0.01 µg L^{-1}	USA	(Hoffman et al., 2000)
	alachlor ethane sulfonic acid	alachlor	< 0.2 - 3.5 µg L^{-1}	0.2 µg L^{-1}	USA	(Kalkhoff et al., 2003)
	alachlor ethane sulfonic acid	alachlor	0.8 - 5.2 µg L^{-1} [c]	0.1 µg L^{-1}	USA	(Kolpin et al., 1996a)
			5.2 - 27.8 µg L^{-1} [b]	0.1 µg L^{-1}	USA	(Kolpin et al., 1996a)

Environmental compartment	Transformation product	Parent pesticide [a]	Concentration	Limit of detection	Country	Reference
stream	alachlor oxanilic acid	alachlor	< 0.2 - 0.54 µg L^{-1}	0.2 µg L^{-1}	USA	(Kalkhoff et al., 2003)
continued…	aldicarb sulfone	aldicarb	ND	0.05 µg L^{-1}	USA	(Hoffman et al., 2000)
	aldicarb sulfoxide	aldicarb	ND	0.05 µg L^{-1}	USA	(Hoffman et al., 2000)
	deethylatrazine	atrazine and propazine	< 0.05 - 0.39 µg L^{-1}	0.05 µg L^{-1}	USA	(Kalkhoff et al., 2003)
			0.04 µg L^{-1} [b]	0.01 µg L^{-1}	USA	(Hoffman et al., 2000)
			23 µg L^{-1} [b]	0.05 µg L^{-1}	USA	(Lerch et al., 1995)
			0.04 µg L^{-1}	0.03 µg L^{-1}	USA	(Battaglin et al., 2009)
	deisopropylatrazine	atrazine, cyanazine and simazine	< 0.05 - 0.36 µg L^{-1}	0.05 µg L^{-1}	USA	(Kalkhoff et al., 2003)
			ND	0.08 µg L^{-1}	USA	(Battaglin et al., 2009)
	hydroxyatrazine	atrazine	< 0.2 - 8.8 µg L^{-1}	0.2 µg L^{-1}	USA	(Kalkhoff et al., 2003)
			0.18 - 5.7 µg L^{-1}	0.04 µg L^{-1}	USA	(Lerch et al., 1995)
			ND	0.032 µg L^{-1}	USA	(Battaglin et al., 2009)
	deethyl hydroxyatrazine	atrazine	<0.12 - 1.9 µg L^{-1}	0.12 µg L^{-1}	USA	(Lerch et al., 1995)
	deisopropyl hydroxyatrazine	atrazine	<0.12 - 0.72 µg L^{-1}	0.12 µg L^{-1}	USA	(Lerch et al., 1995)
	diaminochlorotriazine	atrazine	ND	0.04 µg L^{-1}	USA	(Battaglin et al., 2009)
	cyanazine amide	cyanazine	< 0.05 - 1.2 µg L^{-1}	0.05 µg L^{-1}	USA	(Kalkhoff et al., 2003)
	3-hydroxycarbofuran	carbofuran	ND	0.05 µg L^{-1}	USA	(Hoffman et al., 2000)
	2,4-D methyl ester	2,4-D	ND	0.016 µg L^{-1}	USA	(Battaglin et al., 2009)
	p,p'-DDE	DDT	ND	0.01µg L^{-1}	USA	(Hoffman et al., 2000)
	alpha-HCH	gamma-HCH	ND	0.01 µg L^{-1}	USA	(Hoffman et al., 2000)
	aminomethylphosphonic acid	glyphosate	0.21 µg L^{-1}	0.02 µg L^{-1}	USA	(Battaglin et al., 2009)
	metolachlor ethane sulfonic acid	metolachlor	< 0.2 - 6.7 µg L^{-1}	0.2 µg L^{-1}	USA	(Kalkhoff et al., 2003)
			< 0.2 - 0.57 µg L^{-1}	0.2 µg L^{-1}	USA	(Phillips et al., 1999)
	metolachlor oxanilic acid	metolachlor	< 0.2 - 1.3 µg L^{-1}	0.2 µg L^{-1}	USA	(Kalkhoff et al., 2003)
			< 0.2 - > 0.5 µg L^{-1}	0.2 µg L^{-1}	USA	(Phillips et al., 1999)
	trifluoromethylphenyl urea	fluometuron	ND	0.05 µg L^{-1}	USA	(Coupe et al., 1998)
	deisopropylprometryn	prometryn	ND	0.05 µg L^{-1}	USA	(Coupe et al., 1998)
	3,4-dichloroaniline	propanil	0.9 µg L^{-1}	0.05 µg L^{-1}	USA	(Coupe et al., 1998)
	methomyl	thiodicarb	0.09 ppb	0.04 ppb	USA	(EPA, 1998d)
	triclopyr	triclopyr triethylamine salt	0.64 ppm (sediment)	post-treatment	USA	(EPA, 1998e)
	3,5,6-trichloro-2-pyridinol	triclopyr triethylamine salt	0.06 - 0.18 ppm	1 - 8 hours	USA	(EPA, 1998e)

Environmental compartment	Transformation product	Parent pesticide [a]	Concentration	Limit of detection	Country	Reference
river						
	2,4-dichlorophenol	2,4-D	ND	75 ng L^{-1}	Italy	(Lagana et al., 2002)
	2,4-D methyl ester	2,4-D	ND	0.19 µg L^{-1}	USA	(Battaglin et al., 2009)
	acetochlor oxanilic acid	acetochlor	ND - 0.15 µg L^{-1}	0.01 µg L^{-1}	USA	(Ferrer et al., 1997)
	alachlor ethane sulfonic acid	alachlor	1.55 - 4.75 µg L^{-1} [c]	0.1 µg L^{-1}	USA	(Battaglin and Goolsby, 1999)
			2.1 µg L^{-1}	0.05 µg L^{-1}	USA	(Verstraeten et al., 1999)
	alachlor oxanilic acid	alachlor	ND - 0.21 µg L^{-1}	0.01 µg L^{-1}	USA	(Ferrer et al., 1997)
	2,6-diethylaniline	alachlor	ND - 0.924 µg L^{-1}	5 ng L^{-1}	USA	(Pereira and Rostad, 1990)
	2-chloro-2',6'-diethylacetanilide	alachlor	ND - 0.35 µg L^{-1}	5 ng L^{-1}	USA	(Pereira and Rostad, 1990)
	2-hydroxy-2',6'-diethylacetanilide	alachlor	ND - 0.9 µg L^{-1}	5 ng L^{-1}	USA	(Pereira and Rostad, 1990)
	8-hydroxy-bentazone	bentazone	ND - 27 µg L^{-1}	2 ng L^{-1}	Italy	(Lagana et al., 2002)
	cyanazine amide	cyanazine	0.47 - 0.57 µg L^{-1} [c]	0.05 µg L^{-1}	USA	(Battaglin and Goolsby, 1999)
			0.06 µg L^{-1} [c]	0.02 µg L^{-1}	USA	(Lerch and Blanchard, 2003)
			ND - 222 ng L^{-1}	25 ng L^{-1}	USA	(Pereira and Hostettler, 1993)
	deethylcyanazine	cyanazine	< 0.05 µg L^{-1} [c]	0.05 µg L^{-1}	USA	(Battaglin and Goolsby, 1999)
			ND	0.05 µg L^{-1}	USA	(Verstraeten et al., 1999)
	deethylcyanazine amide	cyanazine	< 0.05 µg L^{-1} [c]	0.5 µg L^{-1}	USA	(Battaglin and Goolsby, 1999)
	deethylatrazine	atrazine and propazine	0.42 - 0.47 µg L^{-1} [c]	0.05 µg L^{-1}	USA	(Battaglin and Goolsby, 1999)
			0.39 - 4.4 µg L^{-1} [b]	0.05 µg L^{-1}	USA	(Thurman et al., 1992)
			ND - 0.407 µg L^{-1}	0.005 µg L^{-1}	Greece	(Albanis et al., 1998)
			ND - 0.215 µg L^{-1}	0.01 µg L^{-1}	Greece	(Albanis and Hela, 1998)
			0.025 - 0.08 µg L^{-1}	0.3 ng L^{-1}	USA	(Sabik et al., 2003)
			7 - 82 ng L^{-1}	5 ng L^{-1}	USA	(Pereira and Rostad, 1990)
			5 - 855 ng L^{-1}	5 ng L^{-1}	USA	(Pereira and Hostettler, 1993)
			150 ng $^{L-1}$ [b]	1 ng L^{-1}	USA	(Liu et al., 2002)
			12 - 28 µg L^{-1} [c]	-	USA	(Solomon et al., 1996)
			ND	0.028 µg L^{-1}	USA	(Battaglin et al., 2009)
			1.7 µg L^{-1} [b]	0.05 µg L^{-}	Canada	(Struger and Fletcher, 2007)
	deisopropylatrazine	atrazine, cyanazine and simazine	0.43 - 0.87 µg L^{-1} [c]	0.05 µg L^{-1}	USA	(Battaglin and Goolsby, 1999)
			< 0.05 - 3.2 µg L^{-1} [b]	0.05 µg L^{-1}	USA	(Thurman et al., 1992)
			0.007 - 0.038 µg L^{-1}	0.3 ng L^{-1}	USA	(Sabik et al., 2003)
			8 - 45 ng L^{-1}	5 ng L^{-1}		(Pereira and Rostad, 1990)
			ND - 335 ng L^{-1}	10 ng L^{-1}	USA	(Pereira and Hostettler, 1993)

Environmental compartment	Transformation product	Parent pesticide [a]	Concentration	Limit of detection	Country	Reference
river			64 ng L^{-1} [b]	1.8 ng L^{-1}	USA	(Liu et al., 2002)
continued…			4.9 - 15 µg L^{-1} [c]	-	USA	(Solomon et al., 1996)
			ND	0.08 µg L^{-1}	USA	(Battaglin et al., 2009)
	hydroxyatrazine	atrazine	ND	0.032 µg L^{-1}	USA	(Battaglin et al., 2009)
	diaminochlorotriazine	atrazine	ND	0.04 µg L^{-1}	USA	(Battaglin et al., 2009)
	p,p'-DDE	DDT	4 ng L^{-1} [b]	0.3 ng L^{-1}	USA	(Liu et al., 2002)
	dimethenamid ethane sulfonic acid	dimethenamid	0.05 µg L^{-1} [c]	0.03 µg L^{-1}	USA	(Zimmerman et al., 2002)
	dimethenamid oxanilic acid	dimethenamid	0.05 µg L^{-1} [c]	0.02 µg L^{-1}	USA	(Zimmerman et al., 2002)
	endosulfan sulphate	endosulfan	6 ng L^{-1}	0.3 ng L^{-1}	USA	(Liu et al., 2002)
	flufenacet ethane sulfonic acid	flufenacet	0.06 µg L^{-1} [c]	0.01 µg L^{-1}	USA	(Zimmerman et al., 2002)
	flufenacet oxanilic acid	flufenacet	0.05 µg L^{-1} [c]	0.07 µg L^{-1}	USA	(Zimmerman et al., 2002)
	aminomethylphosphonic acid	glyphosate	ND	0.02 µg L^{-1}	USA	(Battaglin et al., 2009)
	4-chloro-2-methylphenol	MCPA	ND	50 ng L^{-1}	Italy	(Lagana et al., 2002)
	metolachlor oxanilic acid	metolachlor	ND - 0.29 µg L^{-1}	0.01 µg L^{-1}	USA	(Ferrer et al., 1997)
	metolachlor ethane sulfonic acid	metolachlor	0.33 - 1.82 µg L^{-1}	0.01 µg L^{-1}	USA	(Ferrer et al., 1997)
	3,4-dichloroaniline	propanil	ND - 26 ppb	0.05 ppb	USA	(PSD, 1988)
canal						
	deethylatrazine	atrazine	ND - 0.526	0.01 µg L^{-1}	Greece	(Albanis and Hela, 1998)
			0.03 µg L^{-1}	-	USA	(Battaglin et al., 2009)
	deisopropylatrazine	atrazine, cyanazine and simazine	ND	0.08 µg L^{-1}	USA	(Battaglin et al., 2009)
	2,4-D methyl ester	2,4-D	ND	0.016 µg L^{-1}	USA	(Battaglin et al., 2009)
	aminomethylphosphonic acid	glyphosate	ND	0.02 µg L^{-1}	USA	(Battaglin et al., 2009)
	diaminochlorotriazine	atrazine	ND	0.04 µg L^{-1}	USA	(Battaglin et al., 2009)
pond						
	2,4-D methyl ester	2,4-D	ND	0.19 µg L^{-1}	USA	(Battaglin et al., 2009)
	deethylatrazine	atrazine	0.022 µg L^{-1}	-	USA	(Battaglin et al., 2009)
	deisopropylatrazine	atrazine, cyanazine and simazine	ND	0.08 µg L^{-1}	USA	(Battaglin et al., 2009)
	hydroxyatrazine	atrazine	0.263 µg L^{-1}	0.032 µg L^{-1}	USA	(Battaglin et al., 2009)
	diaminochlorotriazine	atrazine	ND	0.04 µg L^{-1}	USA	(Battaglin et al., 2009)
	aminomethylphosphonic acid	glyphosate	ND	0.02 µg L^{-1}	USA	(Battaglin et al., 2009)

Environmental compartment	Transformation product	Parent pesticide [a]	Concentration	Limit of detection	Country	Reference
lake						
	deethylatrazine	atrazine	1.57 µg L^{-1} [b]	0.05 µg L^{-1}	USA	(Spalding et al., 1994)
			92 ng L^{-1} [c]	2 - 6 ng L^{-1}	Switzerland	(Bucheli et al., 1997)
			0.36 µg L^{-1} [c]	0.05 µg L^{-1}	USA	(Thurman et al., 2000)
			0.18 - 1.57 µg L^{-1} [b] 0.1 - 0.54 µg L^{-1} [c]	0.05 µg L^{-1}	USA	(Spalding et al., 1994)
	deisopropylatrazine	atrazine	1.06 µg L^{-1} [b]	0.09 µg L^{-1}	USA	(Spalding et al., 1994)
			26 ng L^{-1} [c]	2 - 6 ng L^{-1}	Switzerland	(Bucheli et al., 1997)
			ND - 1.06 µg L^{-1} [b] ND - 0.92 µg L^{-1} [c]	0.09 µg L^{-1}	USA	(Spalding et al., 1994)
	hydroxyatrazine	atrazine	0.56 µg L^{-1} [c]	0.05 µg L^{-1}	USA	(Thurman et al., 2000)
	dichlorophenylurea	diuron	0.2 µg L^{-1} [c]	0.2 µg L^{-1}	USA	(Thurman et al., 2000)
	dichloromethylphenylurea	diuron	0.45 µg L^{-1} [c]	0.2 µg L^{-1}	USA	(Thurman et al., 2000)
	3,4-dichloroaniline	diuron	0.31 µg L^{-1} [c]	0.05 µg L^{-1}	USA	(Thurman et al., 2000)
	metolachlor ethane sulfonic acid	metolachlor	0.1 µg L^{-1} [c]	0.2 µg L^{-1}	USA	(Thurman et al., 2000)
	metolachlor oxanilic acid	metolachlor	0.19 µg L^{-1} [c]	0.2 µg L^{-1}	USA	(Thurman et al., 2000)
	demethylnorflurazon	norflurazon	0.17 µg L^{-1} [c]	0.05 µg L^{-1}	USA	(Thurman et al., 2000)
Groundwater						
	3-chloroallyl alcohol	1,3-dichloropropene	trace - 13.5 ppb	0.05 ppb	USA	(EPA, 1998a)
	3-chloroacrylic acid	1,3-dichloropropene	trace - 8.79 ppb	0.05 ppb	USA	(EPA, 1998a)
	2,4-dichlorophenol	2,4-D	4 µg L^{-1} [b]	-	Denmark	(Helweg et al., 2002)
	acetochlor ethane sulfonic acid	acetochlor	0.77 µg L^{-1} [b]	0.2 µg L^{-1}	USA	(Kolpin et al., 2000)
			ND - 3.32 µg L^{-1}	0.2 µg L^{-1}	USA	(Boyd, 2000)
	acetochlor ethane sulfonic acid	acetochlor	0.28 µg L^{-1} [c]	0.1 µg L^{-1}	USA	(Kolpin et al., 1996a)
			8.6 µg L^{-1} [b]	0.1 µg L^{-1}	USA	(Kolpin et al., 1996a)
	acetochlor oxanilic acid	acetochlor	11.5 µg L^{-1} [b]	0.2 µg L^{-1}	USA	(Kolpin et al., 2000)
			ND - 1.75 µg L^{-1}	0.2 µg L^{-1}	USA	(Boyd, 2000)
			ND - 0.17 µg L^{-1}	0.01 µg L^{-1}	USA	(Ferrer et al., 1997)
	α-N-[(2'-6'-diethylphenylamino]ethanol	alachlor	< 2 - 480 ng L^{-1}	-	USA	(Potter and Carpenter, 1995)
	2-chloro-2'-ethyl-6'-ethyl-N-(methoxymethyl)acetanilide	alachlor	< 2 - 310 ng L^{-1}	-	USA	(Potter and Carpenter, 1995)
	2'-acetyl-6'-ethylacetanilide	alachlor	28 - 120 ng L^{-1}	-	USA	(Potter and Carpenter, 1995)

Environmental compartment	Transformation product	Parent pesticide [a]	Concentration	Limit of detection	Country	Reference
Groundwater continued…	2'-acetyl-6'-ethyl-N-methoxymethyl)acetanilide	alachlor	68 - 240 ng L^{-1}	-	USA	(Potter and Carpenter, 1995)
	2-hydroxy-2',6'-diethyl-N-methyl)acetanilide	alachlor	< 2 - 130 ng L^{-1}	-	USA	(Potter and Carpenter, 1995)
	2-hydroxy-2',6'-diethyl-N-methoxymethyl)acetanilide	alachlor	< 2 - 100 ng L^{-1}	-	USA	(Potter and Carpenter, 1995)
	2,6-diethylaniline	alachlor	0.085 µg L^{-1} [b]	0.003 µg L^{-1}	USA	(Kolpin et al., 1998)
			< 2 - 16 ng L^{-1}	-	USA	(Potter and Carpenter, 1995)
			0.02 µg L^{-1} [b]	0.02 µg L^{-1}	USA	(Kolpin et al., 1996b)
	2',6'-diethylacetanilide	alachlor	< 2 - 130 ng L^{-1}	-	USA	(Potter and Carpenter, 1995)
	2',6'-diethylformanilide	alachlor	< 2 - 87 ng L^{-1}	-	USA	(Potter and Carpenter, 1995)
	7-ethylindoline	alachlor	< 2 - 35 ng L^{-1}	-	USA	(Potter and Carpenter, 1995)
	alachlor ethane sulfonic acid	alachlor	1.2 µg L^{-1} [b]	0.05 µg L^{-1}	USA	(Verstraeten et al., 1999)
			8.63 µg L^{-1} [b]	0.1 µg L^{-1}	USA	(Kolpin et al., 1996b)
			8.5 µg L^{-1} [b]	0.2 µg L^{-1}	USA	(Kolpin et al., 2000)
			ND - 2.5 µg L^{-1}	0.2 µg L^{-1}	USA	(Boyd, 2000)
			0.06 - 9.32 µg L^{-1}	0.05 µg L^{-1}	USA	(Aga et al., 1994)
			0.21 - 6.91 µg L^{-1}	-	USA	(EPA, 1998b)
	alachlor oxanilic acid	alachlor	33.4 µg L^{-1} [b]	0.2 µg L^{-1}	USA	(Kolpin et al., 2000)
			ND - 0.31 µg L^{-1}	0.2 µg L^{-1}	USA	(Boyd, 2000)
			0.02 - 1.66 µg L^{-1}	0.01 µg L^{-1}	USA	(Ferrer et al., 1997)
	N-(2,6-diethylphenyl) methylene	alachlor	< 2 - 10 ng L^{-1}	-	USA	(Potter and Carpenter, 1995)
	N-(2,6-diethylphenyl)-N-(methoxymethyl)acetamide	alachlor	100 - 550 ng L^{-1}	-	USA	(Potter and Carpenter, 1995)
	deethylatrazine	atrazine	0.205 µg L^{-1} [b]	1 - 5 ng L^{-1}	Greece	(Albanis et al., 1998)
			0.4 µg L^{-1} [c]	0.04 µg L^{-1}	USA	(Steinheimer and Scoggin, 2001)
			2.32 µg L^{-1} [b]	0.05 µg L^{-1}	USA	(Burkart and Kolpin, 1993)
			7 ng L^{-1}	-	Switzerland	(Bucheli et al., 1997)
			2.6 µg L^{-1} [b]	0.002 µg L^{-1}	USA	(Kolpin et al., 1998)
			5 µg L^{-1}	0.02 µg L^{-1}	USA	(Adams and Thurman, 1991)
			2.2 µg L^{-1} [b]	0.05 µg L^{-1}	USA	(Kolpin et al., 1996b)
			0.59 µg L^{-1} [b]	0.05 µg L^{-1}	USA	(Kolpin et al., 2000)
			ND - 0.44 µg L^{-1}	0.05 µg L^{-1}	USA	(Boyd, 2000)
			0.05 - 0.13 µg L^{-1} [b]	0.02 µg L^{-1}	USA	(Blanchard and Donald, 1997)

Environmental compartment	Transformation product	Parent pesticide [a]	Concentration	Limit of detection	Country	Reference
Groundwater			0.42 µg L^{-1}	-	Australia	(APVMA, 1997)
continued…			1.86 µg L^{-1}	0.05 µg L^{-1}	France	(Baran et al., 2008)
			1.16 µg L^{-1} [b]	0.05 µg L^{-1}	France	(Baran et al., 2007)
			1.03 µg L^{-1}	-	USA	(Steele et al., 2008)
	deisopropylatrazine	atrazine, cyanazine, simazine	0.6 µg L^{-1} [c]	0.04 µg L^{-1}	USA	(Steinheimer and Scoggin, 2001)
			0.16 µg L^{-1}	-	Australia	(APVMA, 1997)
			1.17 µg L^{-1} [b]	0.05 µg L^{-1}	USA	(Kolpin et al., 1996b)
			14 ng L^{-1}	-	Switzerland	(Bucheli et al., 1997)
			< 0.02 µg L^{-1}	0.02 µg L^{-1}	USA	(Adams and Thurman, 1991)
			1.1 µg L^{-1} [b]	0.05 µg L^{-1}	USA	(Kolpin et al., 2000)
			ND - 0.26 µg L^{-1}	0.05 µg L^{-1}	USA	(Boyd, 2000)
			0.36 µg L^{-1} [b]	-	Spain	(Hernandez et al., 2008)
	deisopropylhydroxyatrazine	atrazine, cyanazine, simazine	0.04 µg L^{-1} [c]	0.04 µg L^{-1}	USA	(Steinheimer and Scoggin, 2001)
	hydroxyatrazine	atrazine	1.3 µg L^{-1} [b]	0.2 µg L^{-1}	USA	(Kolpin et al., 2000)
			ND - 0.22 µg L^{-1}	0.2 µg L^{-1}	USA	(Boyd, 2000)
	2-aminobenzimidazole	carbendazim [a]	0.03 µg L^{-1} [b]	-	Spain	(Hernandez et al., 2008)
	3-hydroxy carbofuran	carbofuran	ND	-	Spain	(Hernandez et al., 2008)
	carbofuran-7-PhOH-3CO	carbofuran	0.06	-	Spain	(Hernandez et al., 2008)
	3-carbamyl-1,2,4,5-trichlorobenzoic acid, 3-cyano-6-hydroxy-2,4,5-trichlorobenzamide, 4-hydroxy-2,5,6-trichloroisophthalonitrile and 3-cyano-2,4,5,6-tetrachlorobenzamide combined	chlorothalonil	16 µg L^{-1} [e]	1.5 µg L^{-1}	USA	(EPA, 1999a)
	3-carbamyl-2,4,5-trichlorobenzoic acid	chlorothalonil	trace - 10.1 µg L^{-1}	1.5 µg L^{-1}	USA	(EPA, 1999a)
			2 - 12.6 µg L^{-1}	2 µg L^{-1}	USA	(EPA, 1999a)
			<0.1 - 10.1 µg L^{-1}	-	USA	(EPA, 1999a)
			1.8 - 10.1 µg L^{-1}	-	USA	(EU, 2005)
	3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	chlorothalonil	0.2 µg L^{-1}	1.5 µg L^{-1}	USA	(EPA, 1999a)
			2 - 5 µg L^{-1}	2 µg L^{-1}	USA	(EPA, 1999a)
			<0.2 - 0.2 µg L^{-1}	-		(EPA, 1999a)

Environmental compartment	Transformation product	Parent pesticide [a]	Concentration	Limit of detection	Country	Reference
Groundwater	3-cyano-2,5,6-trichlorobenzamide	chlorothalonil	ND	1.5 µg L^{-1}	USA	(EPA, 1999a)
continued…	4-hydroxy-2,5,6-trichloroisophthalonitrile	chlorothalonil	3.6 µg L^{-1}	2 µg L^{-1}	USA	(EPA, 1999a)
	3-cyano-2,4,5,6-tetrachlorobenzamide	chlorothalonil	2.8 µg L^{-1}	2 µg L^{-1}	USA	(EPA, 1999a)
	3,4-dichloroaniline	chlorpyrifos, diuron, linuron, propanil	<0.025 µg L^{-1} [b]	-	Spain	(Hernandez et al., 2008)
	cyanazine amide	cyanazine	0.55 µg L^{-1} [b]	0.55 µg L^{-1}	USA	(Kolpin et al., 1996b)
			0.64 µg L^{-1} [b]	0.05 µg L^{-1}	USA	(Kolpin et al., 2000)
			ND - 0.31 µg L^{-1}	0.05 µg L^{-1}	USA	(Boyd, 2000)
	deethylcyanazine	cyanazine	ND	0.05 µg L^{-1}	USA	(Verstraeten et al., 1999)
			ND	0.05 µg L^{-1}	USA	(Kolpin et al., 1996b)
	deethylcyanazine amide	cyanazine	ND	0.05 µg L^{-1}	USA	(Kolpin et al., 1996b)
	3,5,6-trichloro-2-pyridinol	chlorpyrifos	ND	50 µg L^{-1}	USA	(EPA, 1999b)
	2-methoxy-3,5,6-trichloropyridine	chlorpyrifos	ND	10 µg L^{-1}	USA	(EPA, 1999b)
	chlorthal-dimethyl mono-acid and di-acid	chlorthal-dimethyl	ND - 158.2 µg L^{-1} [e]	0.05 µg L^{-1}	USA	(Monohan et al., 1995)
	chlorthal-dimethyl di-acid	chlorthal-dimethyl	2.22 µg L^{-1} [b]	0.01 µg L^{-1}	USA	(Kolpin et al., 1996b)
	p,p'-DDE	DDT	0.006 µg L^{-1} [b]	0.006 µg L^{-1}	USA	(Kolpin et al., 1998)
			0.03 µg L^{-1} [b]	0.03 µg L^{-1}	-	(Kolpin et al., 1996b)
	2,6-dichlorbenzamide	diclobenil	180 ppb	-	Netherlands	(EPA, 1998c)
	4-chloroaniline	diflubenzuron	ND	-	Spain	(Hernandez et al., 2008)
	endosulfan sulphate	endosulfan	ND - 1.4 ppb	0.005 ppb	USA	(EPA, 2002)
	AMPA	glyphosate	1.6 µg L^{-1} [b]	-	Denmark	(Helweg et al., 2002)
	α-HCH	gamma-HCH	0.059 µg L^{-1} [b]	0.002 µg L^{-1}	USA	(Kolpin et al., 1998)
	monodesmethyl isoproturon	isoproturon	~0.05 µg L^{-1}	≤ 0.05 µg L^{-1}	France	(Baran et al., 2008)
	didesmethylisoproturon	isoproturon	ND	≤ 0.05 µg L^{-1}	France	(Baran et al., 2008)
	metolachlor ethane sulfonic acid	metolachlor	8.6 µg L^{-1} [b]	0.2 µg L^{-1}	USA	(Kolpin et al., 2000)
			ND - 6.84 µg L^{-1}	0.2 µg L^{-1}	USA	(Boyd, 2000)
			0.1 - 1.83 µg L^{-1}	0.01 µg L^{-1}	USA	(Ferrer et al., 1997)
			15.2 µg L^{-1}	-	USA	(Steele et al., 2008)
	metolachlor oxanilic acid	metolachlor	15.3 µg L^{-1} [b]	0.2 µg L^{-1}	USA	(Kolpin et al., 2000)
			ND - 4.25 µg L^{-1}	0.2 µg L^{-1}	USA	(Boyd, 2000)
			0.03 - 0.91 µg L^{-1}	0.01 µg L^{-1}	USA	(Ferrer et al., 1997)

Environmental compartment	Transformation product	Parent pesticide [a]	Concentration	Limit of detection	Country	Reference
	2,4-bis(isopropylamino)-6-hydroxy-s-triazine	prometryn	0.61 ppb	-	USA	(EPA, 1996)
	hydroxysimazine	simazine	0.15 µg L^{-1} [c]	0.04 µg L^{-1}	USA	(Steinheimer and Scoggin, 2001)
	desethyl-2-hydroxyterbuthylazine	terbuthylazine	0.21 µg L^{-1} [b]	-	Spain	(Hernandez et al., 2008)
	desethylterbuthylazine	terbuthylazine	1.42 µg L^{-1} [b]	-	Spain	(Hernandez et al., 2008)
	hydroxyterbuthylazine	terbuthylazine	0.15 µg L^{-1} [b]	-	Spain	(Hernandez et al., 2008)
	desethylterbumeton	terbumeton	1.62 µg L^{-1} [b]	-	Spain	(Hernandez et al., 2008)
	methomyl	thiodicarb	0.1 -0.4 ppb	-	USA	(EPA, 1998d)
Raw source water						
	hydroxyacetochlor	acetochlor	198 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al., 2006)
	deschloroacetochlor	acetochlor	35 ng L^{-1} [b]	0.07 ng L^{-1}	USA	(Hladik et al, 2006)
	acetochlor oxanilic acid	acetochlor	1170 ng L^{-1} [b]	7 ng L^{-1}	USA	(Hladik et al, 2006)
	acetochlor ethane sulfonic acid	acetochlor	1080 ng L^{-1} [b]	100 ng L^{-1}	USA	(Hladik et al, 2006)
	2-chloro-2'-ethyl-6'-methylacetanilide	acetochlor	167 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	2-hydroxy-2'-ethyl-6'-methylacetanilide	acetochlor	105 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	2-ethyl-6-methylaniline	acetochlor	<25 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	2'-ethyl-6'-methylacetanilide	acetochlor	57 ng L^{-1} [b]	8 ng L^{-1}	USA	(Hladik et al, 2006)
	hydroxyalachlor	alachlor	43 ng L^{-1} [b]	3 ng L^{-1}	USA	(Hladik et al, 2006)
	deschloroalachlor	alachlor	14 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	2-chloro-2'-6'-diethylacetanilide	alachlor	15 ng L^{-1} [b]	0.1 ng L^{-1}	USA	(Hladik et al, 2006)
	2-hydroxy-2'-6'-diethylacetanilide	alachlor	104 ng L^{-1} [b]	0.7 ng L^{-1}	USA	(Hladik et al, 2006)
	2-hydroxy-2'-6'-diethyl-N-methylacetanilide	alachlor	1.7 ng L^{-1} [b]	4 ng L^{-1}	USA	(Hladik et al, 2006)
	2'-6'-diethylacetanilide	alachlor	43 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	2,6-diethylaniline	alachlor	<11 ng L^{-1} [b]	10 ng L^{-1}	USA	(Hladik et al, 2006)
	alachlor oxanilic acid	alachlor	216 ng L^{-1} [b]	7 ng L^{-1}	USA	(Hladik et al, 2006)
	alachlor ethane sulfonic acid	alachlor	945 ng L^{-1} [b]	100 ng L^{-1}	USA	(Hladik et al, 2006)
	deethylatrazine	atrazine	0.14 - 0.24 µg L^{-1} [c]	-	USA	(Solomon et al., 1996)
			0.38 µg L^{-1} [c]	-	USA	(Solomon et al., 1996)
			0.682 µg L^{-1} [b]	-	USA	(Coupe and Blomquist, 2004)
	deethylatrazine continued	atrazine	594 ng L^{-1} [b]	0.3 ng L^{-1}	USA	(Hladik et al, 2006)

Environmental compartment	Transformation product	Parent pesticide [a]	Concentration	Limit of detection	Country	Reference
Raw source water	deisopropylatrazine	atrazine	0.08 - 0.14 µg L^{-1} [c]	-	USA	(Solomon et al., 1996)
continued…			0.1 µg L^{-1} [c]	-	USA	(Solomon et al., 1996)
			199 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	hydroxyatrazine	atrazine	0.8 µg L^{-1} [c]	-	USA	(Solomon et al., 1996)
	azinphos-methyl-oxon	azinphos-methyl	0.263 µg L^{-1} [c]	0.031 µg L^{-1}	USA	(Nguyen et al., 2004)
	o-p'-DDA	DDT	0.28 µg L^{-1}	-	Germany	(Heberer and Dünnbier, 1999)
	p-p'-DDA	DDT	1.7 µg L^{-1}	-	Germany	(Heberer and Dünnbier, 1999)
	deschlorodimethenamid	dimethenamid	14 ng L^{-1} [b]	0.1 ng L^{-1}	USA	(Hladik et al, 2006)
	disulfoton sulfone	disulfoton	0.013 µ g L^{-1} [c]	0.005 µg L^{-1}	USA	(Nguyen et al, 2004)
	disulfoton sulfoxide	disulfoton	0.06 µg L^{-1} [c]	0.016 µg L^{-1}	USA	(Nguyen et al, 2004)
	fenamiphos sulfone	fenamiphos	0.005 µg L^{-1} [c]	0.008 µg L^{-1}	USA	(Nguyen et al, 2004)
	fenamiphos sulfoxide	fenamiphos	0.021 µg L^{-1} [c]	0.008 µg L^{-1}	USA	(Nguyen et al, 2004)
	malaoxon	malathion	ND	0.005 µg L^{-1}	USA	(Nguyen et al, 2004)
	hydroxymetolachlor	metolachlor	217 ng L^{-1} [b]	1 ng L^{-1}	USA	(Hladik et al, 2006)
	deschlorometolachlor	metolachlor	32 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	metolachlor morpholinone	metolachlor	63 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	metolachlor propanol	metolachlor	208 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	deschloroacetylmetachlor	metolachlor	39 ng L^{-1} [b]	0.1 ng L^{-1}	USA	(Hladik et al, 2006)
	deschloroacetyl metachlor propanol	metolachlor	17 ng L^{-1} [b]	0.8 ng L^{-1}	USA	(Hladik et al, 2006)
	metachlor oxanilic acid	metolachlor	687 ng L^{-1} [b]	7 ng L^{-1}	USA	(Hladik et al, 2006)
	metachlor ethane sulfonic acid	metolachlor	1580 ng L^{-1} [b]	90 ng L^{-1}	USA	(Hladik et al, 2006)
Finished drinking water						
	hydroxyacetochlor	acetochlor	64 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	deschloroacetochlor	acetochlor	31 ng L^{-1} [b]	0.07 ng L^{-1}	USA	(Hladik et al, 2006)
	acetochlor oxanilic acid	acetochlor	551 ng L^{-1} [b]	7 ng L^{-1}	USA	(Hladik et al, 2006)
	acetochlor ethane sulfonic acid	acetochlor	845 ng L^{-1} [b]	100 ng L^{-1}	USA	(Hladik et al, 2006)
	2-chloro-2'-ethyl-6'-methylacetanilide	acetochlor	163 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	2-hydroxy-2'-ethyl-6'-methylacetanilide	acetochlor	67 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	2-ethyl-6-methylaniline	acetochlor	<25 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	2'-ethyl-6'-methylacetanilide	acetochlor	57 ng L^{-1} [b]	8 ng L^{-1}	USA	(Hladik et al, 2006)

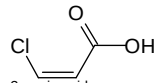
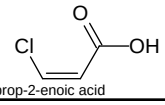
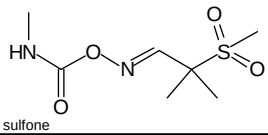
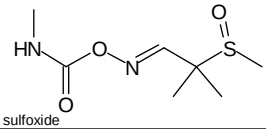
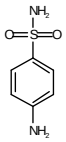
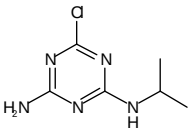
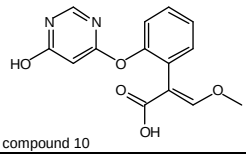
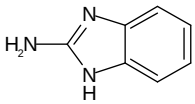
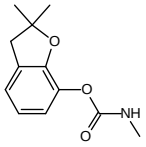
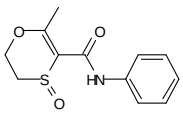
Environmental compartment	Transformation product	Parent pesticide [a]	Concentration	Limit of detection	Country	Reference
Finished drinking water continued…	hydroxyalachlor	alachlor	34 ng L^{-1} [b]	3 ng L^{-1}	USA	(Hladik et al, 2006)
	deschloroalachlor	alachlor	0.7 ng L^{-1} [b]	-	USA	(Hladik et al, 2006)
	2-chloro-2'-6'-diethylacetanilide	alachlor	11 ng L^{-1} [b]	0.1 ng L^{-1}	USA	(Hladik et al, 2006)
	2-hydroxy-2'-6'-diethylacetanilide	alachlor	85 ng L^{-1} [b]	0.7 ng L^{-1}	USA	(Hladik et al, 2006)
	2-hydroxy-2'-6'-diethyl-N-methylacetanilide	alachlor	1.7 ng L^{-1} [b]	4 ng L^{-1}	USA	(Hladik et al, 2006)
	2'-6'-diethylacetanilide	alachlor	38 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	2,6-diethylaniline	alachlor	<11 ng L^{-1} [b]	10 ng L^{-1}	USA	(Hladik et al, 2006)
	alachlor oxanilic acid	alachlor	136 ng L^{-1} [b]	7 ng L^{-1}	USA	(Hladik et al, 2006)
	alachlor ethane sulfonic acid	alachlor	743 ng L^{-1} [b]	100 ng L^{-1}	USA	(Hladik et al, 2006)
	deethylatrazine	atrazine	318 ng L^{-1} [b]	0.3 ng L^{-1}	USA	(Hladik et al, 2006)
			0.352 µg L^{-1} [b]	-	USA	(Coupe and Blomquist, 2004)
	deisopropylatrazine	atrazine	75 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	azinphos-methyl-oxon	azinphos-methyl	0.026 µg L^{-1} [c]	0.031 µg L^{-1}	USA	(Nguyen et al, 2004)
	deschlorodimethenamid	dimethenamid	25 ng L^{-1} [b]	0.1 ng L^{-1}	USA	(Hladik et al, 2006)
	disulfoton sulfone	disulfoton	ND	0.005 µg L^{-1}	USA	(Nguyen et al, 2004)
	disulfoton sulfoxide	disulfoton	ND	0.016 µg L^{-1}	USA	(Nguyen et al, 2004)
	fenamiphos sulfone	fenamiphos	0.011 µg L^{-1} [c]	0.008 µg L^{-1}	USA	(Nguyen et al, 2004)
	fenamiphos sulfoxide	fenamiphos	0.022 µg L^{-1} [c]	0.008 µg L^{-1}	USA	(Nguyen et al, 2004)
	malaoxon	malathion	0.106 µg L^{-1} [c]	0.005 µg L^{-1}	USA	(Nguyen et al, 2004)
	hydroxymetolachlor	metolachlor	61 ng L^{-1} [b]	1 ng L^{-1}	USA	(Hladik et al, 2006)
	deschlorometolachlor	metolachlor	30 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	metolachlor morpholinone	metolachlor	37 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	metolachlor propanol	metolachlor	73 ng L^{-1} [b]	0.2 ng L^{-1}	USA	(Hladik et al, 2006)
	deschloroacetylmetachlor	metolachlor	35 ng L^{-1} [b]	0.1 ng L^{-1}	USA	(Hladik et al, 2006)
	deschloroacetyl metachlor propanol	metolachlor	22 ng L^{-1} [b]	0.8 ng L^{-1}	USA	(Hladik et al, 2006)
	metachlor oxanilic acid	metolachlor	215 ng L^{-1} [b]	7 ng L^{-1}	USA	(Hladik et al, 2006)
	metachlor ethane sulfonic acid	metolachlor	1530 ng L^{-1} [b]	90 ng L^{-1}	USA	(Hladik et al, 2006)

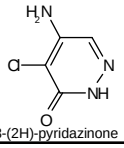
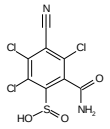
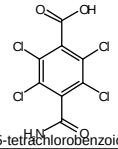
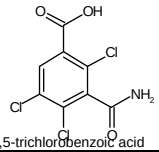
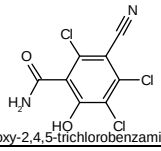
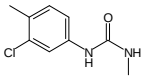
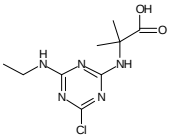
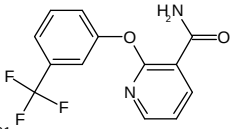
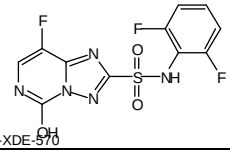
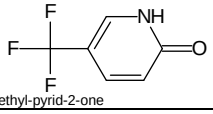
a - pesticide identified in the reference as the source of the transformation product
b - peak concentration during study
c - median or mean concentration
d - calculated average concentration
e - combined transformation product concentration

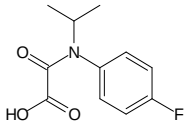
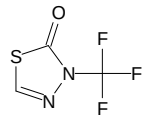
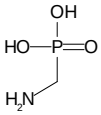
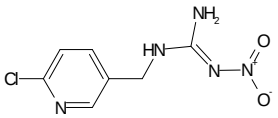
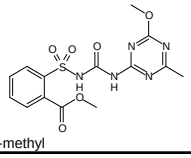
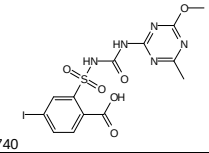
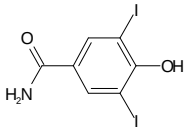
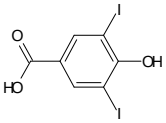
Appendix 5 – DEREK endpoints

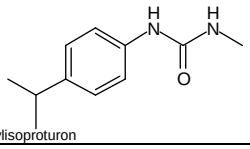
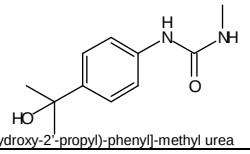
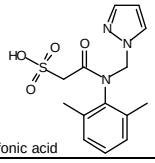
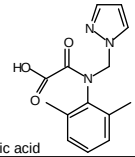
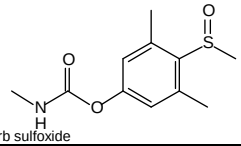
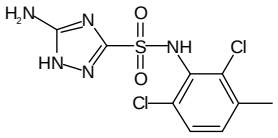
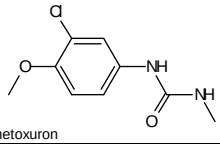
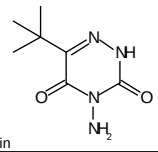
Super end-points	End-points
Carcinogenicity	Carcinogenicity Photocarcinogenicity
Chromosome damage	Chromosome damage Photo-induced chromosome damage
Genotoxicity	Genotoxicity Photogenotoxicity
Hepatotoxicity	Hepatotoxicity
HERG channel inhibition	HERG channel inhibition
Irritation	Irritation (of the eye) Irritation (of the gastrointestinal tract) Irritation (of the respiratory tract) Irritation (of the skin) Lachrymation
Miscellaneous endpoints	alpha-2-mu-Globulin nephropathy Anaphylaxis Anticholinesterase activity Bladder urothelial hyperplasia Cerebral oedema Chloracne Cumulative effect on white cell count and immunology Cyanide-type effects High acute toxicity Methaemoglobinaemia Neurotoxicity Ocular toxicity Oestrogenicity Peroxisome proliferation Phospholipidosis Phototoxicity Pulmonary toxicity Uncoupler of oxidative phosphorylation
Mutagenicity	Mutagenicity Photomutagenicity
Reproductive toxicity	Developmental toxicity Teratogenicity Testicular toxicity
Respiratory Sensitisation	Occupational asthma Respiratory sensitisation
Skin Sensitisation	Photoallergenicity Skin sensitisation
Thyroid Toxicity	Thyroid toxicity

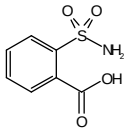
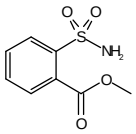
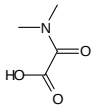
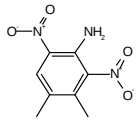
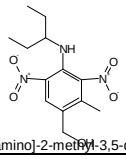
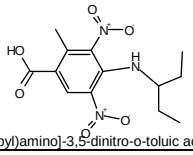
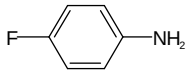
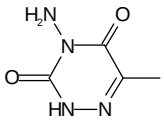
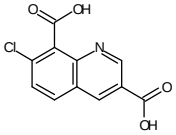
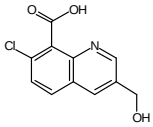
Appendix 6 – Metabolites selected for further study

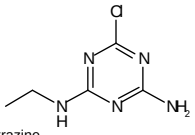
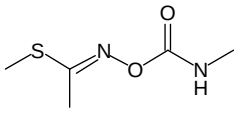
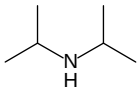
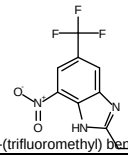
Metabolite code	Parent pesticide	Metabolite name and structure	Reasoning (estimated to be…)	Expsoure rank out of 485	Mammalian metabolite
3	1,3-dichloropropene	 cis-3-chloroprop-2-enoic acid	carcinogen & mutagen	6	no
5	1,3-dichloropropene	 trans-3-chloroprop-2-enoic acid	carcinogen & mutagen	7	no
14	aldicarb	 aldicarb sulfone	developmental toxicant & pesticidaly activ	14	yes
15	aldicarb	 aldicarb sulfoxide	potent LD50 & pesticidaly active	33	yes
25	asulam	 sulfanilamide	thyroid toxicity	121	yes
26	atrazine	 deethylatrazine	pesticidaly active	66	yes
33	azoxystrobin	 reference compound 10	developmental toxicant	30	no
61	Carbendazim	 2-aminobenzimidazole	mutagen	22	yes
63	carbosulfan	 carbofuran	potent LD50	176	yes
65	Carboxin	 carboxin sulfoxide	pesticidaly active	41	yes

Metabolite code	Parent pesticide	Metabolite name and structure	Reasoning (estimated to be…)	Expsoure rank out of 485	Mammalian metabolite
67	chloridazon	 5-amino-4-chloro-3-(2H)-pyridazinone	mutagen	32	no
75	chlorothalonil	 R417888	carcinogen & developmental toxicant	1	no
69	chlorothalonil	 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	carcinogen & developmental toxicant	3	no
70	chlorothalonil	 3-carbamyl-2,4,5-trichlorobenzoic acid	carcinogen & developmental toxicant	5	no
73	chlorothalonil	 3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	potent LD50	57	no
76	chlorotoluron	 3-(3-chloro-p-tolyl)-1-methylurea	pesticidaly active	24	yes
84	cyanazine	 cyanazine acid	pesticidaly active	10	yes
114	diflufenican	 AE 0542291	carcinogen & developmental toxicant	15	no
166	florasulam	 5-hydroxy-XDE-570	pesticidaly active	77	yes
169	fluazifop-P-butyl	 5-trifluoromethyl-pyrid-2-one	potent LD50	90	no data

Metabolite code	Parent pesticide	Metabolite name and structure	Reasoning (estimated to be…)	Expsoure rank out of 485	Mammalian metabolite
174	flufenacet	 FOE oxalte	carcinogen	8	no
176	flufenacet	 thiadone	carcinogen & potent LD50	17	yes
191	fosetyl-aluminium	 phosphorous acid	pesticidaly active	11	yes
190	fosetyl-aluminium	 ethanol	teratogenicity	108	yes
199	glyphosate	 aminomethylphosphonic acid	potent LD50	177	yes
205	imidacloprid	 1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	mutagen	51	no
211	iodosulfuron-methyl	 metsulfuron-methyl	pesticidaly active	89	yes
210	iodosulfuron-methyl	 AE F145740	thyroid toxicity	230	yes
212	ioxynil	 3,5-diiodo-4-hydroxybenzamide	thyroid toxicity	404	no
213	ioxynil	 3,5-diiodo-4-hydroxybenzoic acid	thyroid toxicity	448	no

Metabolite code	Parent pesticide	Metabolite name and structure	Reasoning (estimated to be…)	Expsoure rank out of 485	Mammalian metabolite
218	isoproturon	 desmethylisoproturon	carcinogen & pesticidaly active	18	no
217	isoproturon	 3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	pesticidaly active	23	yes
255	metaldehyde	 acetaldehyde	mutagen	29	yes
259	metazachlor	 metazachlor sulfonic acid	developmental toxicant	2	no
258	metazachlor	 metazachlor oxalic acid	developmental toxicant	9	no
266	methiocarb	 methiocarb sulfoxide	potent LD50	103	yes
270	metosulam	 ATSA	thyroid toxicity	418	no
271	Metoxuron	 demethyl metoxuron	potent LD50	97	no data
276	metribuzin	 diketo metribuzin	teratogenicity & pesticidaly active	69	no

Metabolite code	Parent pesticide	Metabolite name and structure	Reasoning (estimated to be…)	Expsoure rank out of 485	Mammalian metabolite
279	metsulfuron-methyl	 IN-D5119	teratogenicity	94	no
280	metsulfuron-methyl	 IN-D5803	teratogenicity	168	yes
295	oxamyl	 dimethyloxamic acid	carcinogen & developmental toxicant	13	yes
299	pendimethalin	 2,6-dinitro-3,4-xylidine	mutagen	19	no
300	pendimethalin	 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcc	mutagen	40	yes
301	pendimethalin	 4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid	mutagen	59	yes
304	picolinafen	 4-fluoroaniline	mutagen	47	yes
349	pymetrozine	 CGA 294849	teratogenicity	158	no
358	quinmerac	 BH518-2	pesticidaly active	37	yes
359	quinmerac	 BH518-4	pesticidaly active	80	yes

Metabolite code	Parent pesticide	Metabolite name and structure	Reasoning (estimated to be…)	Expsoure rank out of 485	Mammalian metabolite
368	simazine	 deisopropylatrazine	pesticidaly active	63	no
416	thiodicarb	 methomyl	potent LD50	114	yes
442	Tri-allate	 diisopropylamine	developmental toxicant	21	no
470	trifluralin	 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	potent LD50	140	no

Appendix 7 – Detailed GIS processing methodology

Adapted from the Fera (CSL) project report for the Defra project 'Design of a targeted mitigation system for transport of pesticides in drainflow in the UK' (Project number PS2218)

Calculation of areas for each crop/soil combination within the catchments

Areas covered by individual soil series and crops had to be calculated for each catchment to underpin the modelling tool. A spatial analysis was undertaken using the following methodology.

A7.1 Spatial analysis

The methodology that was used to derive areas for each crop/soil combination within a catchment is described below. This is illustrated with an example in Section A7.2. All spatial processing was performed in ArcDesktop 9.1 using geoprocessing scripts written in PYTHON.

1. A spatial intersect of the LCM/soil/ward maps resulted in polygons. All polygons that were located outside the catchment boundaries were ignored. There is some uncertainty associated with the identification of land cover class using satellite imagery. Areas per LCM class based on the agricultural survey data are more accurate than those in the LCM 2000 database. The areas within each polygon were, therefore, adjusted using a correction factor specific to each ward and LCM class:

   The areas for the crops reported in the agricultural statistics were first aggregated by LCM 2000 class. This was done separately for each ward. The calculated total area assigned to a particular class within a ward was divided by the total area of that class reported in the LCM 2000 database. This ratio was used to correct the polygon areas. This ensured that the total area cultivated with a particular crop within each ward corresponds to that from the agricultural survey.

2. The corrected total area within each LCM/soil/ward polygon was allocated to individual crops. This was based on ward-specific proportions of individual crops within each LCM class calculated from the agricultural survey data. The proportions were calculated as the ratio of the area covered by each individual crop (e.g. wheat) allocated to a LCM 2000 class (e.g. 4.1) and the total area for all crops in that class.

   Within each ward, it was assumed that the various crops are evenly distributed across all polygons with the same land cover. This is unlikely to be the case in reality, but a spatially more accurate method could not be employed given the resolution of the available data.

3. The calculated areas within all polygons were summed by crop and soil association to give the total area for each crop/soil combination within the whole catchment.

4. The area for each combination of crop and soil association was divided into that for individual soil series using the expected percentage of each series within the association from the NATMAP associations table. Total areas for each combination of crop and soil series were then calculated.

A7.2 Processing steps (example)

1. The total area of each LCM class (4.1, 4.2, 4.3) for each ward was quantified

Ward	LCM class	LCM_total(ha)
48619	4.1 arable cereals	914

2. Crop data from the agricultural survey for each ward was aggregated into the appropriate LCM class (i.e. area cultivated with wheat + barley + oats + maize + other cereals)

Ward	Area of LCM class 4.1 (ha)
48619	1195

3. The total area from step 2 was divided by the total area from step 1

Ward	LCM class	Ratio
48619	4.1	1.31

4. The ward-specific proportion of each crop within the LCM 2000 class was calculated.

		Land cover class 4.1						
Ward		Wheat	Winter barley	Spring barley	Maize	Oats	Other cereals	Total
48619	Area (ha)	986	95	0	40	74		1195
	%	82.3	8.0	0	3.4	6.2		100.00

5. A spatial intersect of soil/LCM/ward/catchment was performed

6. The polygons created by the intersect were totalled for each soil type per LCM class per ward and multiplied by the ratio calculated in step 3.

Ward	Soil Association	Class	Total area (ha)	Ratio	Final area (ha)
48619	Denchworth	4.1	22.7	1.31	29.6

7. The areas derived in step 6 where then multiplied by the proportion of each crop from step 4 (according to ward) and added over all wards the catchment boundaries. This gave the area of crop grown on each soil association in each catchment.

Catchment	Soil Association	Crop	Total Area (ha)
Catchment A	Denchworth	Wheat	5000.6

8. The areas derived in step 7 where divided into the various soil series. Each series may occur in more than one association. Areas cultivated with the same crop on the same soil series were added over all associations. This resulted in the total area of crop grown on each soil series.

Catchment	Soil Association	Crop	Total area (ha)	% Denchworth	Area (ha)
Catchment A	Denchworth	Wheat	5000.6	38	1900.2
Catchment A	Elmton 3	Wheat	298.8	19	56.8
Catchment A	Evesham 2	Wheat	532.0	35	186.2
Catchment A	Oxpasture	Wheat	628.9	12	75.5
Catchment A	Wickham 2	Wheat	2910.7	17	494.8

Total 2730.5

A7.3 Results

Tables A7.1-A7.3 show the areas for the most dominant combinations of soil series and crop in Catchment A, Catchment B and Catchment C. Wheat is the most important crop in all three catchments, followed by oilseed rape in Catchment A and Catchment B and spring barley in Catchment C. The total area covered with agricultural crops in Catchment A (32676 ha) corresponds to 36% of the total area (91479 ha). The remaining land includes grassland, forests, lakes, roads and urban developments. In Catchment B and Catchment C, 44% and 31% of the total area, respectively, is used for crops.

Table A7.1 Areas (ha) covered by the most dominant soil series and crops in Catchment A

Soil series	WHEAT	OILSEED	WBARLEY	FIELDBEAN	SETASIDE	OATS	OTHER	SUM
WICKHAM	2821	1059	412	477	149	95	62	5073
DENCHWORTH	2713	963	386	455	196	113	53	4880
BANBURY	2078	567	387	351	75	53	103	3613
ABERFORD	1806	588	411	241	4	43	84	3177
OXPASTURE	1528	564	223	260	73	62	35	2746
EVESHAM	1429	487	205	227	75	56	25	2506
OTHER	5998	1896	1068	874	471	146	227	10681
Total	18374	6125	3094	2884	1042	569	589	32676

Table A7.2 Areas (ha) covered by the most dominant soil series and crops in Catchment B

Soil series	WHEAT	OILSEED	FIELDBEAN	WBARLEY	OATS	LINSEED	OTHER	SUM
DENCHWORTH	2163	645	348	264	179	9	21	3629
WICKHAM	1387	488	226	163	113	10	10	2397
EVESHAM	1306	367	225	165	124	5	20	2212
OXPASTURE	917	330	147	113	66	6	4	1583
WHIMPLE	694	208	122	109	27	0	12	1172
LAWFORD	625	184	99	74	46	2	4	1035
OTHER	2291	887	407	371	92	13	38	4098
Total	9383	3110	1573	1259	648	45	110	16127

Table A7.3 Areas (ha) covered by the most dominant soil series and crops in Catchment C

Soil series	WHEAT	SBARLEY	SETASIDE	OILSEED	WBARLEY	OATS	OTHER	SUM
ANDOVER	5374	2391	1301	1114	943	509	759	12390
UPTON	2792	981	616	587	596	225	504	6302
PANHOLES	1664	740	423	353	300	138	256	3873
COOMBE	1578	680	408	351	293	113	259	3682
CHARITY	1557	629	432	336	276	155	257	3641
ICKNIELD	1255	485	485	357	260	93	272	3206
OTHER	8903	2667	2239	2361	1523	686	2143	20521
Total	23123	8572	5904	5458	4191	1918	4451	53616

Appendix 8 – Estimated metabolite concentrations in downstream surface waters for three selected catchments

Table A8.1. Estimated metabolite concentrations (µg/L) in downstream surface waters for Catchment A

Metabolite code	Metabolite	Parent pesticide	Conservative Estimate	Refined Estimate I (DT50 refinement)	Refined Estimate II (Pesticide usage refinement)
3	cis-3-chloroprop-2-enoic acid	1,3-dichloropropene	<0.01	<0.01	<0.01
5	trans-3-chloroprop-2-enoic acid	1,3-dichloropropene	<0.01	<0.01	<0.01
14	aldicarb sulfone	Aldicarb	<0.01	<0.01	<0.01
15	aldicarb sulfoxide	Aldicarb	<0.01	<0.01	<0.01
25	sulfanilamide	Asulam	<0.01	<0.01	<0.01
26	deethylatrazine	Atrazine	<0.01	<0.01	<0.01
33	reference compound 10	Azoxystrobin	1.10	0.76	<0.01
61	2-aminobenzimidazole	Carbendazim	7.50	4.64	<0.01
63	carbofuran	Carbosulfan	<0.01	<0.01	<0.01
65	carboxin sulfoxide	Carboxin	1.61	0.92	<0.01
67	5-amino-4-chloropyridazin-3(2H)-one	Chloridazon	<0.01	<0.01	<0.01
69	3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	Chlorothalonil	17.12	12.71	0.04
70	3-carbamyl-2,4,5-trichlorobenzoic acid	Chlorothalonil	6.67	6.67	0.02
73	3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	Chlorothalonil	0.80	0.72	<0.01
75	R417888	Chlorothalonil	29.48	29.48	0.07
76	3-(3-chloro-p-tolyl)-1-methylurea	Chlorotoluron	6.52	5.87	<0.01
84	cyanazine acid	Cyanazine	16.55	7.47	<0.01
114	AE 0542291	Diflufenican	2.22	1.82	<0.01
166	5-hydroxy-XDE-570	Florasulam	0.09	0.09	<0.01
169	5-trifluoromethyl-pryid-2-one	Fluazifop-P-butyl	1.92	1.11	<0.01
174	FOE oxalte	Flufenacet	1.70	0.86	0.03
176	thiadone	Flufenacet	0.49	0.31	0.01
190	ethanol	Fosetyl-aluminium	<0.01	<0.01	<0.01
191	phosphorous acid	Fosetyl-aluminium	<0.01	<0.01	<0.01
199	aminomethylphosphonic acid	Glyphosate	0.64	0.64	0.01
205	1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	Imidacloprid	0.22	0.09	0.01
210	AE F145740	Iodosulfuron-methyl	0.01	0.01	<0.01
211	metsulfuron-methyl	Iodosulfuron-methyl	0.11	0.11	<0.01
212	3,5-di-iodo-4-hydroxybenzamide	Ioxynil	0.28	0.28	<0.01
213	3,5-di-iodo-4-hydroxybenzoic acid	Ioxynil	0.61	0.61	<0.01
217	3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	Isoproturon	0.52	0.35	0.03
218	desmethylisoproturon	Isoproturon	1.11	1.11	0.06
255	acetaldehyde	Metaldehyde	4.82	2.10	0.01
258	metazachlor oxalic acid	Metazachlor	3.96	2.60	0.12
259	metazachlor sulfonic acid	Metazachlor	10.27	5.10	0.31
266	methiocarb sulfoxide	Methiocarb	3.37	2.12	0.04

Metabolite code	Metabolite	Parent pesticide	Conservative Estimate	Refined Estimate I (DT50 refinement)	Refined Estimate II (Pesticide usage refinement)
271	demethyl metoxuron	Metoxuron	<0.01	<0.01	<0.01
276	diketo metribuzin	Metribuzin	<0.01	<0.01	<0.01
279	IN-D5119	Metsulfuron-methyl	0.08	0.04	<0.01
280	IN-D5803	Metsulfuron-methyl	0.02	0.02	<0.01
295	dimethyloxamic acid	Oxamyl	<0.01	<0.01	<0.01
299	2,6-dinitro-3,4-xylidine	Pendimethalin	1.01	0.89	0.03
300	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	Pendimethalin	0.45	0.50	0.01
301	4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid	Pendimethalin	0.31	0.39	0.01
304	4-fluoroaniline	Picolinafen	0.09	0.06	<0.01
349	CGA 294849	Pymetrozine	<0.01	<0.01	<0.01
358	BH518-2	Quinmerac	0.24	0.24	0.01
359	BH518-4	Quinmerac	0.06	0.04	<0.01
368	deisopropylatrazine	Simazine	0.32	0.21	<0.01
416	methomyl	Thiodicarb	13.10	13.10	<0.01
442	diisopropylamine	Tri-allate	21.08	14.2	<0.01
470	2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	Trifluralin	0.28	0.31	<0.01

Table A8.2. Estimated metabolite concentrations (µg/L) in downstream surface waters for Catchment B

Metabolite code	Metabolite	Parent pesticide	Conservative Estimate	Refined Estimate I (DT50 refinement)	Refined Estimate II (Pesticide usage refinement)
3	cis-3-chloroprop-2-enoic acid	1,3-dichloropropene	16.81	8.78	<0.01
5	trans-3-chloroprop-2-enoic acid	1,3-dichloropropene	16.81	8.78	<0.01
14	aldicarb sulfone	Aldicarb	16.01	3.63	<0.01
15	aldicarb sulfoxide	Aldicarb	0.70	0.43	<0.01
25	sulfanilamide	Asulam	0.02	0.01	<0.01
26	deethylatrazine	Atrazine	0.01	0.01	0.11
33	reference compound 10	Azoxystrobin	2.19	1.33	<0.01
61	2-aminobenzimidazole	Carbendazim	14.20	7.68	0.01
63	carbofuran	Carbosulfan	0.02	0.02	<0.01
65	carboxin sulfoxide	Carboxin	3.17	1.61	<0.01
67	5-amino-4-chloropyridazin-3(2H)-one	Chloridazon	0.02	0.02	<0.01
69	3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	Chlorothalonil	33.83	22.02	0.04
70	3-carbamyl-2,4,5-trichlorobenzoic acid	Chlorothalonil	12.45	12.45	0.01
73	3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	Chlorothalonil	1.61	1.26	<0.01
75	R417888	Chlorothalonil	53.25	53.25	0.06
76	3-(3-chloro-p-tolyl)-1-methylurea	Chlorotoluron	13.03	10.33	0.12
84	cyanazine acid	Cyanazine	30.70	11.92	<0.01

Metabolite code	Metabolite	Parent pesticide	Conservative Estimate	Refined Estimate I (DT50 refinement)	Refined Estimate II (Pesticide usage refinement)
114	AE 0542291	Diflufenican	4.44	3.19	0.03
166	5-hydroxy-XDE-570	Florasulam	0.14	0.14	<0.01
169	5-trifluoromethyl-pryid-2-one	Fluazifop-P-butyl	3.23	1.65	<0.01
174	FOE oxalte	Flufenacet	3.27	1.44	0.12
176	thiadone	Flufenacet	0.97	0.54	0.04
190	ethanol	Fosetyl-aluminium	0.06	0.03	<0.01
191	phosphorous acid	Fosetyl-aluminium	4.48	1.15	0.23
199	aminomethylphosphonic acid	Glyphosate	1.15	1.15	0.01
205	1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	Imidacloprid	0.44	0.15	0.03
210	AE F145740	Iodosulfuron-methyl	0.01	0.01	<0.01
211	metsulfuron-methyl	Iodosulfuron-methyl	0.17	0.17	<0.01
212	3,5-di-iodo-4-hydroxybenzamide	Ioxynil	0.43	0.43	<0.01
213	3,5-di-iodo-4-hydroxybenzoic acid	Ioxynil	0.88	0.88	<0.01
217	3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	Isoproturon	1.01	0.59	0.27
218	desmethylisoproturon	Isoproturon	1.93	1.93	0.52
255	acetaldehyde	Metaldehyde	9.18	3.48	0.04
258	metazachlor oxalic acid	Metazachlor	7.18	4.05	0.64
259	metazachlor sulfonic acid	Metazachlor	18.09	7.68	1.61
266	methiocarb sulfoxide	Methiocarb	6.62	3.67	0.09
271	demethyl metoxuron	Metoxuron	0.04	0.04	<0.01
276	diketo metribuzin	Metribuzin	0.08	0.05	<0.01
279	IN-D5119	Metsulfuron-methyl	0.14	0.06	<0.01
280	IN-D5803	Metsulfuron-methyl	0.03	0.03	<0.01
295	dimethyloxamic acid	Oxamyl	2.21	0.77	<0.01
299	2,6-dinitro-3,4-xylidine	Pendimethalin	2.04	1.58	0.27
300	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	Pendimethalin	0.92	0.91	0.12
301	4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid	Pendimethalin	0.64	0.70	0.09
304	4-fluoroaniline	Picolinafen	0.17	0.10	<0.01
349	CGA 294849	Pymetrozine	0.03	0.01	<0.01
358	BH518-2	Quinmerac	0.36	0.36	0.01
359	BH518-4	Quinmerac	0.11	0.07	<0.01
368	deisopropylatrazine	Simazine	0.66	0.38	<0.01
416	methomyl	Thiodicarb	23.14	23.14	<0.01
442	diisopropylamine	Tri-allate	41.37	24.57	<0.01
470	2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	Trifluralin	0.57	0.56	0.01

Table A8.3. Estimated metabolite concentrations (µg/L) in downstream surface waters for Catchment C

Metabolite code	Metabolite	Parent pesticide	Conservative Estimate	Refined Estimate I (DT50 refinement)	Refined Estimate II (Pesticide usage refinement)
3	cis-3-chloroprop-2-enoic acid	1,3-dichloropropene	0.03	<0.01	<0.01
5	trans-3-chloroprop-2-enoic acid	1,3-dichloropropene	0.03	<0.01	<0.01
14	aldicarb sulfone	Aldicarb	0.19	0.06	<0.01
15	aldicarb sulfoxide	Aldicarb	0.01	0.01	<0.01
25	sulfanilamide	Asulam	<0.01	<0.01	0.01
26	deethylatrazine	Atrazine	<0.01	<0.01	<0.01
33	reference compound 10	Azoxystrobin	0.04	0.02	<0.01
61	2-aminobenzimidazole	Carbendazim	0.25	0.11	<0.01
63	carbofuran	Carbosulfan	<0.01	<0.01	<0.01
65	carboxin sulfoxide	Carboxin	0.07	0.03	<0.01
67	5-amino-4-chloropyridazin-3(2H)-one	Chloridazon	<0.01	<0.01	<0.01
69	3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	Chlorothalonil	0.63	0.32	<0.01
70	3-carbamyl-2,4,5-trichlorobenzoic acid	Chlorothalonil	0.21	0.21	<0.01
73	3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	Chlorothalonil	0.03	0.02	<0.01
75	R417888	Chlorothalonil	0.82	0.82	<0.01
76	3-(3-chloro-p-tolyl)-1-methylurea	Chlorotoluron	0.32	0.19	<0.01
84	cyanazine acid	Cyanazine	0.30	0.10	<0.01
114	AE 0542291	Diflufenican	0.09	0.05	<0.01
166	5-hydroxy-XDE-570	Florasulam	<0.01	<0.01	<0.01
169	5-trifluoromethyl-pryid-2-one	Fluazifop-P-butyl	0.07	0.03	<0.01
174	FOE oxalte	Flufenacet	0.06	0.02	<0.01
176	thiadone	Flufenacet	0.02	0.01	<0.01
190	ethanol	Fosetyl-aluminium	<0.01	<0.01	<0.01
191	phosphorous acid	Fosetyl-aluminium	0.12	0.04	<0.01
199	aminomethylphosphonic acid	Glyphosate	0.03	0.03	<0.01
205	1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	Imidacloprid	0.01	<0.01	<0.01
210	AE F145740	Iodosulfuron-methyl	<0.01	<0.01	<0.01
211	metsulfuron-methyl	Iodosulfuron-methyl	<0.01	<0.01	<0.01
212	3,5-di-iodo-4-hydroxybenzamide	Ioxynil	0.01	0.01	<0.01
213	3,5-di-iodo-4-hydroxybenzoic acid	Ioxynil	0.01	0.01	<0.01
217	3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	Isoproturon	0.02	0.01	<0.01
218	desmethylisoproturon	Isoproturon	0.04	0.04	<0.01
255	acetaldehyde	Metaldehyde	0.17	0.05	<0.01
258	metazachlor oxalic acid	Metazachlor	0.10	0.04	<0.01
259	metazachlor sulfonic acid	Metazachlor	0.23	0.08	<0.01
266	methiocarb sulfoxide	Methiocarb	0.14	0.06	<0.01
271	demethyl metoxuron	Metoxuron	<0.01	<0.01	<0.01
276	diketo metribuzin	Metribuzin	<0.01	<0.01	<0.01
279	IN-D5119	Metsulfuron-methyl	<0.01	<0.01	<0.01

Metabolite code	Metabolite	Parent pesticide	Conservative Estimate	Refined Estimate I (DT50 refinement)	Refined Estimate II (Pesticide usage refinement)
280	IN-D5803	Metsulfuron-methyl	<0.01	<0.01	<0.01
295	dimethyloxamic acid	Oxamyl	0.01	<0.01	<0.01
299	2,6-dinitro-3,4-xylidine	Pendimethalin	0.05	0.03	<0.01
300	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	Pendimethalin	0.02	0.02	<0.01
301	4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid	Pendimethalin	0.02	0.01	<0.01
304	4-fluoroaniline	Picolinafen	<0.01	<0.01	<0.01
349	CGA 294849	Pymetrozine	<0.01	<0.01	<0.01
358	BH518-2	Quinmerac	<0.01	<0.01	<0.01
359	BH518-4	Quinmerac	<0.01	<0.01	<0.01
368	deisopropylatrazine	Simazine	0.01	<0.01	<0.01
416	methomyl	Thiodicarb	0.38	0.38	<0.01
442	diisopropylamine	Tri-allate	0.88	0.41	<0.01
470	2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	Trifluralin	0.01	0.01	<0.01

Appendix 9 – Parameters utilised during the estimation of removal by ozone and chlorine

Table A9.1. Removal via ozonation grouped by percent removal range and properties

Removal via Ozonation	Metabolite		% removal via		Parameter Ozone Correlation				
	Name	Code	Ozone	Chlorine[a]	SASA	#rtvFG	PISA	WPSA	#metab
90-100%	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	26	100	89	536.835	0	35.079	0	6
	metazachlorsulfonicacid	2	100	75	526.65	0	232.604	2.098	4
	2;6-dinitro-3;4-xylidine	16	96	82	406.115	0	50.017	0	5
	4-[(1-ethylpropyl)amino]-3;5-dinitro-o-toluic acid	31	95	87	534.675	0	34.616	0	5
	carboxin sulfoxide	27	91	69	479.039	0	205.04	20.911	3
80-89%	metazachlor oxalic acid	8	86	78	481.428	2	226.344	0	3
	methiocarb sulfoxide	41	85	58	479.084	0	65.452	14.742	3
	metsulfuron-methyl	37	82	66	636.674	1	181.221	0.307	2
	5-hydroxy-XDE-570	35	82	66	523.791	0	224.436	104.776	3
	sulfanilamide	44	80	69	351.899	0	149.354	1.938	1
70-79%	BH518-4	36	79	67	432.291	0	165.603	59.659	2
	reference compound 10	22	79	73	493.139	1	215.916	0	1
	desmethylisoproturon	15	79	63	471.928	0	129.263	0	1
	carbofuran	48	79	56	463.104	0	128.489	0	1
	3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	19	79	66	477.685	0	124.724	0	1
	2-aminobenzimidazole	18	77	71	322.041	0	200.899	0	0
	aminomethylphosphonic acid	49	77	55	259.946	0	0	2.938	2
	4-fluoroaniline	28	76	65	289.818	0	182.147	47.014	1
	1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	29	76	55	447.664	0	125.294	74.652	2
	IN-D5119	39	75	45	365.979	0	156.604	1.653	0
	AE F145740	50	75	68	634.164	0	135.711	83.869	2
	5-amino-4-chloro-3-(2H)-pyridazinone	23	73	61	294.694	0	57.486	63.514	2
	ATSA	52	73	74	492.259	0	101.178	89.913	2
	diisopropylamine	17	73	34	340.033	0	0	0	1
	AE 0542291	13	72	46	471.284	0	254.437	113.966	1
	3-(3-chloro-p-tolyl)-1-methyl urea	20	72	55	434.676	0	117.864	61.027	1
	IN-D5803	47	71	41	407.271	1	156.84	1.775	0
	aldicarbsulfoxide	24	70	46	456.756	0	0	17.898	1
	demethyl metoxuron	40	70	56	450.877	0	110.554	71.28	1
60-69%	BH518-2	25	69	64	429.413	0	165.907	59.315	0
	CGA 294849	46	68	57	313	1	6.012	0	1
	2-ethyl-7-nitro-5(trifluoromethyl) benzimidazole	45	68	46	460.769	0	80.726	117.289	2
	ethanol	42	68	55	19.118	1	0	0	1
	aldicarbsulfone	12	67	39	469.135	0	0	0	0
	5-trifluoromethyl-pryid2-one	38	65	35	321.322	0	111.139	118.256	1
	3,5-di-iodo-4hydroxybenzamide	51	64	53	389.644	0	80.157	158.703	2
	diketometribuzin	34	63	54	384.77	1	0.572	0	0
	3-cyano-6-hydroxy-2;4;5-trichlorobenzamide	30	61	51	412.381	0	47.01	168.674	2
	R417888	1	60	52	441.061	0	42.475	176.679	2
50-59%	FOEoxalte	7	59	53	433.801	2	119.008	47.008	0
	dimethyloxamicacid	11	59	48	301.505	2	0	0	0
	3,5-di-iodo-4hydroxybenzoic acid	53	59	52	384.243	0	80.926	158.703	1
	methomyl	43	58	46	410.448	1	0	41.692	0
	cis-3-chloroprop-2-enoicacid	5	57	31	263.841	1	31.487	69.25	0
	trans-3-chloroprop-2-enoicacid	6	57	31	263.841	1	31.487	69.25	0
	desisopropylatrazine	32	56	48	378.037	1	31.463	76.218	0
	desethylatrazine	33	56	47	402.048	1	29.319	76.218	0
	cyanaiznechloroacid	9	56	59	506.369	1	26.629	76.211	0
	thiadone	14	50	6	301.698	0	52.752	173.413	0

[a] – for comparison

Table A9.2. Removal via chlorination grouped by percent removal range and properties

Removal via Chlorine	Metabolite		% removal via		Parameter Chlorine Correlation				
	Name	Code	Chlorine	Ozone[a]	SASA	#rtvFG	FISA	QPlog Po/w	IP(eV)
80-89%	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	26	89	100	536.835	0	198.141	1.767	9.362
	4-[(1-ethylpropyl)amino]-3;5-dinitro-o-toluic acid	31	87	95	534.675	0	242.016	1.902	9.741
	2;6-dinitro-3;4-xylidine	16	82	96	406.115	0	216.89	0.631	9.407
70-79%	metazachloroxalic acid	8	78	86	481.428	2	127.886	1.915	9.6
	metazachlorsulfonic acid	2	75	100	526.65	0	144.98	1.625	9.605
	ATSA	52	74	73	492.259	0	221.539	0.724	8.859
	reference compound 10	22	73	79	493.139	1	188.111	1.055	9.453
	2-aminobenzimidazole	18	71	77	322.041	0	121.142	1	8.209
60-69%	sulfanilamide	44	69	80	351.899	0	200.606	-0.794	8.785
	carboxin sulfoxide	27	69	91	479.039	0	59.852	1.369	8.45
	carboxin sulfoxide	50	68	75	634.164	0	230.442	1.319	9.647
	BH518-4	36	67	79	432.291	0	154.35	1.559	9.158
	5-hydroxy-XDE-570	35	66	82	523.791	0	194.579	0.849	9.573
	metsulfuron-methyl	37	66	82	636.674	1	199.21	0.84	10.172
	3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	19	66	79	477.685	0	111.751	1.279	8.602
	4-fluoroaniline	28	65	76	289.818	0	60.657	1.802	8.127
	BH518-2	25	64	69	429.413	0	204.191	1.618	9.401
	desmethylisoproturon	15	63	79	471.928	0	78.193	1.907	8.6
	5-amino-4-chloro-3-(2H)-pyridazinone	23	61	73	294.694	0	173.693	-0.364	8.844
50-59%	cyanazine acid	9	59	56	506.369	1	151.079	1.925	9.017
	methiocarb sulfoxide	41	58	85	479.084	0	98.533	0.964	9.153
	CGA 294849	46	57	68	313	1	218.572	-0.796	9.719
	demethyl metoxuron	40	56	70	450.877	0	78.238	1.592	8.499
	carbofuran	48	56	79	463.104	0	55.462	2.317	8.951
	ethanol	42	55	68	19.118	1	0	-2.209	6.855
	aminomethylphosphonic acid	49	55	77	259.946	0	203.447	-3.167	8.89
	1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	29	55	76	447.664	0	213.243	0.228	10.02
	3-(3-chloro-p-tolyl)-1-methylurea	20	55	72	434.676	0	77.343	1.727	8.707
	diketometribuzin	34	54	63	384.77	1	189.81	0.322	9.691
	3,5-di-iodo-4hydroxybenzamide	51	53	64	389.644	0	150.784	1.116	9.039
	FOEoxalte	7	53	59	433.801	2	124.816	1.8	9.792
	R417888	1	52	60	441.061	0	221.907	0.38	9.504
	3,5-di-iodo-4hydroxybenzoic acid	53	52	59	384.243	0	144.615	1.964	9.065
	3-cyano-6-hydroxy-2;4;5-trichlorobenzamide	30	51	61	412.381	0	196.696	1.151	9.612
40-49%	dimethyloxamicacid	11	48	59	301.505	2	145.465	-0.287	9.673
	desisopropylatrazine	32	48	56	378.037	1	129.507	0.399	9.038
	desethylatrazine	33	47	56	402.048	1	120.708	0.809	9.092
	2-ethyl-7-nitro-5(trifluoromethyl) benzimidazole	45	46	68	460.769	0	122.901	2.602	9.926
	aldicarbsulfoxide	24	46	70	456.756	0	131.985	-0.347	9.199
	AE 0542291	13	46	72	471.284	0	102.172	2.525	9.878
	methomyl	43	46	58	410.448	1	99.58	1.025	9.171
	IN-D5119	39	45	75	365.979	0	207.721	-0.343	10.393
	IN-D5803	47	41	71	407.271	1	163.508	-0.113	10.507
30-39%	aldicarb sulfone	12	39	67	469.135	0	167.518	-0.532	9.852
	5-trifluoromethyl-pryid2-one	38	35	65	321.322	0	91.283	1.192	9.665
	diisopropylamine	17	34	73	340.033	0	8.972	1.157	9.172
	cis-3-chloroprop-2-enoic acid	5	31	57	263.841	1	109.222	0.791	10.105
	trans-3-chloroprop-2-enoic acid	6	31	57	263.841	1	109.222	0.791	10.105
0-9%	thiadone	14	6	50	301.698	0	74.686	0.611	10.403

[a] – for comparison

Appendix 10 – Estimated metabolite concentrations in finished drinking waters for three selected catchments

Table A10.1. Estimated metabolite concentrations (µg/L) in finished drinking waters for Catchment A

Metabolite code	Metabolite	Parent pesticide	Conservative Estimate			Refined Estimate II (Pesticide usage refinement)		
			Influent	Conventional	Advanced	Influent	Conventional	Advanced
3	cis-3-chloroprop-2-enoic acid	1,3-dichloropropene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
5	trans-3-chloroprop-2-enoic acid	1,3-dichloropropene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
14	aldicarb sulfone	Aldicarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
15	aldicarb sulfoxide	Aldicarb	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
25	sulphanilamide	Asulam	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
26	deethylatrazine	Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
33	reference compound 10	Azoxystrobin	1.10	0.11	0.02	<0.01	<0.01	<0.01
61	2-aminobenzimidazole	Carbendazim	7.50	1.09	0.25	<0.01	<0.01	<0.01
63	carbofuran	Carbosulfan	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
65	carboxin sulfoxide	Carboxin	1.61	0.32	0.03	<0.01	<0.01	<0.01
67	5-amino-4-chloropyridazin-3(2H)-one	Chloridazon	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
69	3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	Chlorothalonil	17.12	8.35 [a]	8.35 [a]	0.04	0.02 [a]	0.02 [a]
70	3-carbamyl-2,4,5-trichlorobenzoic acid	Chlorothalonil	6.67	2.50 [a]	2.50 [a]	0.02	0.01 [a]	0.01 [a]
73	3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	Chlorothalonil	0.80	0.15	0.06	<0.01	<0.01	<0.01
75	R417888	Chlorothalonil	29.48	12.00	4.84	0.07	0.03	0.01
76	3-(3-chloro-p-tolyl)-1-methylurea	Chlorotoluron	6.52	0.74	0.16	<0.01	<0.01	<0.01
84	cyanazine acid	Cyanazine	16.55	1.69	0.75	<0.01	<0.01	<0.01
114	AE 0542291	Diflufenican	2.22	0.30	0.08	<0.01	<0.01	<0.01
166	5-hydroxy-XDE-570	Florasulam	0.09	0.01	<0.01	<0.01	<0.01	<0.01
169	5-trifluoromethyl-pryid-2-one	Fluazifop-P-butyl	1.92	0.63	0.22	<0.01	<0.01	<0.01
174	FOE oxalte	Flufenacet	1.70	0.30	0.12	0.03	0.01	<0.01
176	thiadone	Flufenacet	0.49	0.23	0.12	0.01	<0.01	<0.01
190	ethanol	Fosetyl-aluminium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
191	phosphorous acid	Fosetyl-aluminium	<0.01	<0.01 [a]	<0.01 [a]	<0.01	<0.01 [a]	<0.01 [a]
199	aminomethylphosphonic acid	Glyphosate	0.64	0.18	0.04	0.01	<0.01	<0.01
205	1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	Imidacloprid	0.22	0.09	0.02	0.01	<0.01	<0.01
210	AE F145740	Iodosulfuron-methyl	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
211	metsulfuron-methyl	Iodosulfuron-methyl	0.11	0.01	<0.01	<0.01	<0.01	<0.01

Metabolite code	Metabolite	Parent pesticide	Conservative Estimate			Refined Estimate II (Pesticide usage refinement)		
			Influent	Conventional	Advanced	Influent	Conventional	Advanced
212	3,5-di-iodo-4-hydroxybenzamide	Ioxynil	0.28	0.05	0.02	<0.01	<0.01	<0.01
213	3,5-di-iodo-4-hydroxybenzoic acid	Ioxynil	0.61	0.02	0.01	<0.01	<0.01	<0.01
217	3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	Isoproturon	0.52	0.09	0.02	0.03	<0.01	<0.01
218	desmethylisoproturon	Isoproturon	1.11	0.10	0.02	0.06	0.01	<0.01
255	acetaldehyde	Metaldehyde	4.82	4.10 [a]	4.10 [a]	0.01	0.01 [a]	0.01 [a]
258	metazachlor oxalic acid	Metazachlor	3.96	0.43	0.06	0.12	0.01	<0.01
259	metazachlor sulfonic acid	Metazachlor	10.27	1.69	0.02	0.31	0.05	<0.01
266	methiocarb sulfoxide	Methiocarb	3.37	0.70	0.11	0.04	0.01	<0.01
271	demethyl metoxuron	Metoxuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
276	diketo metribuzin	Metribuzin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
279	IN-D5119	Metsulfuron-methyl	0.08	0.02	0.01	<0.01	<0.01	<0.01
280	IN-D5803	Metsulfuron-methyl	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
295	dimethyloxamic acid	Oxamyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
299	2,6-dinitro-3,4-xylidine	Pendimethalin	1.01	0.34	0.14	0.03	0.01	<0.01
300	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	Pendimethalin	0.45	0.02	<0.01	0.01	<0.01	<0.01
301	4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid	Pendimethalin	0.31	<0.01	<0.01	0.01	<0.01	<0.01
304	4-fluoroaniline	Picolinafen	0.09	<0.01	<0.01	<0.01	<0.01	<0.01
349	CGA 294849	Pymetrozine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
358	BH518-2	Quinmerac	0.24	0.09	0.03	0.01	<0.01	<0.01
359	BH518-4	Quinmerac	0.06	<0.01	<0.01	<0.01	<0.01	<0.01
368	deisopropylatrazine	Simazine	0.32	0.04	0.01	<0.01	<0.01	<0.01
416	methomyl	Thiodicarb	13.10	3.41	1.50	<0.01	<0.01	<0.01
442	diisopropylamine	Tri-allate	21.08	7.47	3.12	<0.01	<0.01	<0.01
470	2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	Trifluralin	0.28	0.09	0.03	<0.01	<0.01	<0.01

[a] – estimation of removal due to chlorination and/or ozonation not possible for this molecule

Table A10.2. Estimated metabolite concentrations (µg/L) in finished drinking waters for Catchment B

Metabolite code	Metabolite	Parent pesticide	Conservative Estimate			Refined Estimate II (Pesticide usage refinement)		
			Influent	Conventional	Advanced	Influent	Conventional	Advanced
3	cis-3-chloroprop-2-enoic acid	1,3-dichloropropene	16.81	5.67	2.51	<0.01	<0.01	<0.01
5	trans-3-chloroprop-2-enoic acid	1,3-dichloropropene	16.81	5.67	2.51	<0.01	<0.01	<0.01
14	aldicarb sulfone	Aldicarb	16.01	8.30	2.74	<0.01	<0.01	<0.01
15	aldicarb sulfoxide	Aldicarb	0.70	0.32	0.10	<0.01	<0.01	<0.01
25	sulphanilamide	Asulam	0.02	0.01	<0.01	<0.01	<0.01	<0.01
26	deethylatrazine	Atrazine	0.01	<0.01	<0.01	0.11	0.02	0.01
33	reference compound 10	Azoxystrobin	2.19	0.22	0.05	<0.01	<0.01	<0.01
61	2-aminobenzimidazole	Carbendazim	14.20	2.07	0.46	0.01	<0.01	<0.01
63	carbofuran	Carbosulfan	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
65	carboxin sulfoxide	Carboxin	3.17	0.64	0.06	<0.01	<0.01	<0.01
67	5-amino-4-chloropyridazin-3(2H)-one	Chloridazon	0.02	0.01	<0.01	<0.01	<0.01	<0.01
69	3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	Chlorothalonil	33.83	16.49 [a]	16.49 [a]	0.04	0.02 [a]	0.02 [a]
70	3-carbamyl-2,4,5-trichlorobenzoic acid	Chlorothalonil	12.45	4.67 [a]	4.67 [a]	0.01	0.01 [a]	0.01 [a]
73	3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	Chlorothalonil	1.61	0.30	0.12	<0.01	<0.01	<0.01
75	R417888	Chlorothalonil	53.25	21.68	8.74	0.06	0.02	0.01
76	3-(3-chloro-p-tolyl)-1-methylurea	Chlorotoluron	13.03	1.48	0.31	0.12	0.01	<0.01
84	cyanazine acid	Cyanazine	30.70	3.14	1.39	<0.01	<0.01	<0.01
114	AE 0542291	Diflufenican	4.44	0.60	0.17	0.03	<0.01	<0.01
166	5-hydroxy-XDE-570	Florasulam	0.14	0.02	<0.01	<0.01	<0.01	<0.01
169	5-trifluoromethyl-pryid-2-one	Fluazifop-P-butyl	3.23	1.06	0.37	<0.01	<0.01	<0.01
174	FOE oxalte	Flufenacet	3.27	0.57	0.24	0.12	0.02	0.01
176	thiadone	Flufenacet	0.97	0.45	0.23	0.04	0.02	0.01
190	ethanol	Fosetyl-aluminium	0.06	0.02	0.01	<0.01	<0.01	<0.01
191	phosphorous acid	Fosetyl-aluminium	4.48	3.81 [a]	3.81 [a]	0.23	0.20 [a]	0.20 [a]
199	aminomethylphosphonic acid	Glyphosate	1.15	0.33	0.07	0.01	<0.01	<0.01
205	1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	Imidacloprid	0.44	0.17	0.04	0.03	0.01	<0.01
210	AE F145740	Iodosulfuron-methyl	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
211	metsulfuron-methyl	Iodosulfuron-methyl	0.17	0.02	<0.01	<0.01	<0.01	<0.01

Metabolite code	Metabolite	Parent pesticide	Conservative Estimate			Refined Estimate II (Pesticide usage refinement)		
			Influent	Conventional	Advanced	Influent	Conventional	Advanced
212	3,5-di-iodo-4-hydroxybenzamide	Ioxynil	0.43	0.08	0.03	<0.01	<0.01	<0.01
213	3,5-di-iodo-4-hydroxybenzoic acid	Ioxynil	0.88	0.03	0.01	<0.01	<0.01	<0.01
217	3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	Isoproturon	1.01	0.17	0.04	0.27	0.05	0.01
218	desmethylisoproturon	Isoproturon	1.93	0.18	0.04	0.52	0.05	0.01
255	acetaldehyde	Metaldehyde	9.18	7.80 [a]	7.80 [a]	0.04	0.03 [a]	0.03 [a]
258	metazachlor oxalic acid	Metazachlor	7.18	0.77	0.11	0.64	0.07	0.01
259	metazachlor sulfonic acid	Metazachlor	18.09	2.97	0.03	1.61	0.27	<0.01
266	methiocarb sulfoxide	Methiocarb	6.62	1.38	0.21	0.09	0.02	<0.01
271	demethyl metoxuron	Metoxuron	0.08	0.02	0.01	<0.01	<0.01	<0.01
276	diketo metribuzin	Metribuzin	0.14	0.04	0.02	<0.01	<0.01	<0.01
279	IN-D5119	Metsulfuron-methyl	0.03	0.01	<0.01	<0.01	<0.01	<0.01
280	IN-D5803	Metsulfuron-methyl	2.21	0.64	0.19	<0.01	<0.01	<0.01
295	dimethyloxamic acid	Oxamyl	2.04	0.68	0.28	0.27	0.09	0.04
299	2,6-dinitro-3,4-xylidine	Pendimethalin	0.92	0.04	<0.01	0.12	0.01	<0.01
300	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	Pendimethalin	0.64	0.01	<0.01	0.09	<0.01	<0.01
301	4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid	Pendimethalin	0.17	<0.01	<0.01	<0.01	<0.01	<0.01
304	4-fluoroaniline	Picolinafen	0.03	<0.01	<0.01	<0.01	<0.01	<0.01
349	CGA 294849	Pymetrozine	0.36	0.13	0.04	0.01	<0.01	<0.01
358	BH518-2	Quinmerac	0.11	0.01	<0.01	<0.01	<0.01	<0.01
359	BH518-4	Quinmerac	0.66	0.08	0.02	<0.01	<0.01	<0.01
368	deisopropylatrazine	Simazine	23.14	6.03	2.66	<0.01	<0.01	<0.01
416	methomyl	Thiodicarb	41.37	14.66	6.13	<0.01	<0.01	<0.01
442	diisopropylamine	Tri-allate	0.57	0.19	0.05	0.01	<0.01	<0.01
470	2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	Trifluralin	0.50	0.02	0.01	0.01	<0.01	<0.01

[a] – estimation of removal due to chlorination and/or ozonation not possible for this molecule

Table A10.3. Estimated metabolite concentrations (µg/L) in finished drinking waters for Catchment C

Metabolite code	Metabolite	Parent pesticide	Conservative Estimate			Refined Estimate II (Pesticide usage refinement)		
			Influent	Conventional	Advanced	Influent	Conventional	Advanced
3	cis-3-chloroprop-2-enoic acid	1,3-dichloropropene	0.03	0.01	<0.01	<0.01	<0.01	<0.01
5	trans-3-chloroprop-2-enoic acid	1,3-dichloropropene	0.03	0.01	<0.01	<0.01	<0.01	<0.01
14	aldicarb sulfone	Aldicarb	0.19	0.10	0.03	<0.01	<0.01	<0.01
15	aldicarb sulfoxide	Aldicarb	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
25	sulphanilamide	Asulam	<0.01	<0.01	<0.01	0.01	<0.01	<0.01
26	deethylatrazine	Atrazine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
33	reference compound 10	Azoxystrobin	0.04	<0.01	<0.01	<0.01	<0.01	<0.01
61	2-aminobenzimidazole	Carbendazim	0.25	0.04	0.01	<0.01	<0.01	<0.01
63	carbofuran	Carbosulfan	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
65	carboxin sulfoxide	Carboxin	0.07	0.01	<0.01	<0.01	<0.01	<0.01
67	5-amino-4-chloropyridazin-3(2H)-one	Chloridazon	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
69	3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	Chlorothalonil	0.63	0.31 [a]	0.31 [a]	<0.01	<0.01 [a]	<0.01 [a]
70	3-carbamyl-2,4,5-trichlorobenzoic acid	Chlorothalonil	0.21	0.08 [a]	0.08 [a]	<0.01	<0.01 [a]	<0.01 [a]
73	3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	Chlorothalonil	0.03	0.01	<0.01	<0.01	<0.01	<0.01
75	R417888	Chlorothalonil	0.82	0.33	0.13	<0.01	<0.01	<0.01
76	3-(3-chloro-p-tolyl)-1-methylurea	Chlorotoluron	0.32	0.04	0.01	<0.01	<0.01	<0.01
84	cyanazine acid	Cyanazine	0.30	0.03	0.01	<0.01	<0.01	<0.01
114	AE 0542291	Diflufenican	0.09	0.01	<0.01	<0.01	<0.01	<0.01
166	5-hydroxy-XDE-570	Florasulam	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
169	5-trifluoromethyl-pryid-2-one	Fluazifop-P-butyl	0.07	0.02	0.01	<0.01	<0.01	<0.01
174	FOE oxalte	Flufenacet	0.06	0.01	<0.01	<0.01	<0.01	<0.01
176	thiadone	Flufenacet	0.02	0.01	<0.01	<0.01	<0.01	<0.01
190	ethanol	Fosetyl-aluminium	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
191	phosphorous acid	Fosetyl-aluminium	0.12	0.10 [a]	0.10 [a]	<0.01	<0.01 [a]	<0.01 [a]
199	aminomethylphosphonic acid	Glyphosate	0.03	0.01	<0.01	<0.01	<0.01	<0.01
205	1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	Imidacloprid	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
210	AE F145740	Iodosulfuron-methyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Metabolite code	Metabolite	Parent pesticide	Conservative Estimate			Refined Estimate II (Pesticide usage refinement)		
			Influent	Conventional	Advanced	Influent	Conventional	Advanced
211	metsulfuron-methyl	Iodosulfuron-methyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
212	3,5-di-iodo-4-hydroxybenzamide	Ioxynil	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
213	3,5-di-iodo-4-hydroxybenzoic acid	Ioxynil	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
217	3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	Isoproturon	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
218	desmethylisoproturon	Isoproturon	0.04	<0.01	<0.01	<0.01	<0.01	<0.01
255	acetaldehyde	Metaldehyde	0.17	0.14 [a]	0.14 [a]	<0.01	<0.01 [a]	<0.01 [a]
258	metazachlor oxalic acid	Metazachlor	0.10	0.01	<0.01	<0.01	<0.01	<0.01
259	metazachlor sulfonic acid	Metazachlor	0.23	0.04	<0.01	<0.01	<0.01	<0.01
266	methiocarb sulfoxide	Methiocarb	0.14	0.03	<0.01	<0.01	<0.01	<0.01
271	demethyl metoxuron	Metoxuron	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
276	diketo metribuzin	Metribuzin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
279	IN-D5119	Metsulfuron-methyl	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
280	IN-D5803	Metsulfuron-methyl	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
295	dimethyloxamic acid	Oxamyl	0.05	0.02	0.01	<0.01	<0.01	<0.01
299	2,6-dinitro-3,4-xylidine	Pendimethalin	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
300	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	Pendimethalin	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
301	4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid	Pendimethalin	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
304	4-fluoroaniline	Picolinafen	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
349	CGA 294849	Pymetrozine	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
358	BH518-2	Quinmerac	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
359	BH518-4	Quinmerac	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
368	deisopropylatrazine	Simazine	0.38	0.10	0.04	<0.01	<0.01	<0.01
416	methomyl	Thiodicarb	0.88	0.31	0.13	<0.01	<0.01	<0.01
442	diisopropylamine	Tri-allate	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
470	2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	Trifluralin	0.50	0.02	0.01	0.01	<0.01	<0.01

[a] – estimation of removal due to chlorination and/or ozonation not possible for this molecule

Appendix 11 – Calculated intakes of pesticide metabolites from indirect exposure through drinking water consumption for three selected catchments

Table A11.1. Total indirect adult exposure to pesticide metabolites for Catchment A

Metabolite code	Pesticide Metabolite	ADI[1] (µg/kg bw/day)	Total Daily Intake (µg/kg bw/day) Conservative Estimate		%ADI Conservative Estimate		Total Daily Intake (µg/kg bw/day) Refined Estimate II		%ADI Refined Estimate II	
			Conventional	Advanced	Conventional	Advanced	Conventional	Advanced	Conventional	Advanced
3	cis-3-chloroprop-2-enoic acid	12.5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
5	trans-3-chloroprop-2-enoic acid	12.5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
14	aldicarb sulfone	1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
15	aldicarb sulfoxide	1.25	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
25	sulfanilamide	360	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
26	deethylatrazine	20	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
33	reference compound 10	100	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
61	2-aminobenzimidazole	20	0.03	0.01	0.16	0.04	<0.01	<0.01	<0.01	<0.01
63	carbofuran	1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
65	carboxin sulfoxide	1.6	0.01	<0.01	0.58	0.06	<0.01	<0.01	<0.01	<0.01
67	5-amino-4-chloropyridazin-3(2H)-one	100	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
69	3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	18	0.24	0.24	1.33	1.33	<0.01	<0.01	<0.01	<0.01
70	3-carbamyl-2,4,5-trichlorobenzoic acid	500	0.07	0.07	0.01	0.01	<0.01	<0.01	<0.01	<0.01
73	3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	5	<0.01	<0.01	0.08	0.03	<0.01	<0.01	<0.01	<0.01
75	R417888	60	0.34	0.14	0.57	0.23	<0.01	<0.01	<0.01	<0.01
76	3-(3-chloro-p-tolyl)-1-methylurea	40	0.02	<0.01	0.05	0.01	<0.01	<0.01	<0.01	<0.01
84	cyanazine acid	2	0.05	0.02	2.42	1.07	<0.01	<0.01	<0.01	<0.01
114	AE 0542291	200	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
166	5-hydroxy-XDE-570	50	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
169	5-trifluoromethyl-pryid-2-one	10	0.02	0.01	0.18	0.06	<0.01	<0.01	<0.01	<0.01
174	FOE oxalte	4	0.01	<0.01	0.21	0.09	<0.01	<0.01	<0.01	<0.01
176	thiadone	4	0.01	<0.01	0.17	0.08	<0.01	<0.01	<0.01	<0.01
190	ethanol	24	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
191	phosphorous acid	3900	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
199	aminomethylphosphonic acid	300	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
205	1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	60	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
210	AE F145740	30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
211	metsulfuron-methyl	250	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
212	3,5-di-iodo-4-hydroxybenzamide	50	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Metabolite code	Pesticide Metabolite	ADI[1] (µg/kg bw/day)	Total Daily Intake (µg/kg bw/day) Conservative Estimate		%ADI Conservative Estimate		Total Daily Intake (µg/kg bw/day) Refined Estimate II		%ADI Refined Estimate II	
213	3,5-di-iodo-4-hydroxybenzoic acid	50	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
217	3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	15	<0.01	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
218	desmethylisoproturon	15	<0.01	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
255	acetaldehyde	20	0.12	0.12	0.59	0.59	<0.01	<0.01	<0.01	<0.01
258	metazachlor oxalic acid	80	0.01	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
259	metazachlor sulfonic acid	80	0.05	<0.01	0.06	<0.01	<0.01	<0.01	<0.01	<0.01
266	methiocarb sulfoxide	13	0.02	<0.01	0.15	0.02	<0.01	<0.01	<0.01	<0.01
270	ATSA	5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
271	demethyl metoxuron	740	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
276	diketo metribuzin	13	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
279	IN-D5119	250	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
280	IN-D5803	250	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
295	dimethyloxamic acid	1	0.01	<0.01	0.96	0.40	<0.01	<0.01	0.03	0.01
299	2,6-dinitro-3,4-xylidine	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
300	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
301	4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
304	4-fluoroaniline	14	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
349	CGA 294849	30	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
358	BH518-2	79	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
359	BH518-4	79	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
368	deisopropylatrazine	5	0.10	0.04	1.95	0.86	<0.01	<0.01	<0.01	<0.01
416	methomyl	2.5	0.21	0.09	8.53	3.57	<0.01	<0.01	<0.01	<0.01
442	diisopropylamine	25	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
470	2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table A11.2. Total indirect toddler exposure to pesticide metabolites for Catchment A

Metabolite code	Pesticide Metabolite	ADI[1] (µg/kg bw/day)	Total Daily Intake (µg/kg bw/day) Conservative Estimate		%ADI Conservative Estimate		Total Daily Intake (µg/kg bw/day) Refined Estimate II		%ADI Refined Estimate II	
			Conventional	Advanced	Conventional	Advanced	Conventional	Advanced	Conventional	Advanced
3	cis-3-chloroprop-2-enoic acid	12.5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
5	trans-3-chloroprop-2-enoic acid	12.5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
14	aldicarb sulfone	1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
15	aldicarb sulfoxide	1.25	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
25	sulfanilamide	360	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
26	deethylatrazine	20	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
33	reference compound 10	100	0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
61	2-aminobenzimidazole	20	0.14	0.03	0.71	0.16	<0.01	<0.01	<0.01	<0.01
63	carbofuran	1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
65	carboxin sulfoxide	1.6	0.04	<0.01	2.62	0.26	<0.01	<0.01	<0.01	<0.01
67	5-amino-4-chloropyridazin-3(2H)-one	100	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
69	3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	18	1.08	1.08	6.00	6.00	<0.01	<0.01	0.01	0.01
70	3-carbamyl-2,4,5-trichlorobenzoic acid	500	0.32	0.32	0.06	0.06	<0.01	<0.01	<0.01	<0.01
73	3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	5	0.02	0.01	0.38	0.15	<0.01	<0.01	<0.01	<0.01
75	R417888	60	1.55	0.63	2.59	1.04	<0.01	<0.01	0.01	<0.01
76	3-(3-chloro-p-tolyl)-1-methylurea	40	0.10	0.02	0.24	0.05	<0.01	<0.01	<0.01	<0.01
84	cyanazine acid	2	0.22	0.10	**10.95**	4.86	<0.01	<0.01	<0.01	<0.01
114	AE 0542291	200	0.04	0.01	0.02	0.01	<0.01	<0.01	<0.01	<0.01
166	5-hydroxy-XDE-570	50	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
169	5-trifluoromethyl-pryid-2-one	10	0.08	0.03	0.81	0.29	<0.01	<0.01	<0.01	<0.01
174	FOE oxalte	4	0.04	0.02	0.97	0.40	<0.01	<0.01	0.02	0.01
176	thiadone	4	0.03	0.01	0.75	0.37	<0.01	<0.01	0.01	0.01
190	ethanol	24	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
191	phosphorous acid	3900	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
199	aminomethylphosphonic acid	300	0.02	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
205	1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	60	0.01	<0.01	0.02	<0.01	<0.01	<0.01	<0.01	<0.01
210	AE F145740	30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
211	metsulfuron-methyl	250	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
212	3,5-di-iodo-4-hydroxybenzamide	50	0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Metabolite code	Pesticide Metabolite	ADI[1] (µg/kg bw/day)	Total Daily Intake (µg/kg bw/day) Conservative Estimate		%ADI Conservative Estimate		Total Daily Intake (µg/kg bw/day) Refined Estimate II		%ADI Refined Estimate II	
213	3,5-di-iodo-4-hydroxybenzoic acid	50	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
217	3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	15	0.01	<0.01	0.08	0.02	<0.01	<0.01	<0.01	<0.01
218	desmethylisoproturon	15	0.01	<0.01	0.09	0.02	<0.01	<0.01	<0.01	<0.01
255	acetaldehyde	20	0.53	0.53	2.65	2.65	<0.01	<0.01	0.01	0.01
258	metazachlor oxalic acid	80	0.06	0.01	0.07	0.01	<0.01	<0.01	<0.01	<0.01
259	metazachlor sulfonic acid	80	0.22	<0.01	0.27	<0.01	0.01	<0.01	0.01	<0.01
266	methiocarb sulfoxide	13	0.09	0.01	0.70	0.11	<0.01	<0.01	0.01	<0.01
270	ATSA	5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
271	demethyl metoxuron	740	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
276	diketo metribuzin	13	<0.01	<0.01	0.02	0.01	<0.01	<0.01	<0.01	<0.01
279	IN-D5119	250	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
280	IN-D5803	250	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
295	dimethyloxamic acid	1	0.04	0.02	4.36	1.81	<0.01	<0.01	0.12	0.05
299	2,6-dinitro-3,4-xylidine	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
300	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
301	4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
304	4-fluoroaniline	14	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
349	CGA 294849	30	0.01	<0.01	0.04	0.01	<0.01	<0.01	<0.01	<0.01
358	BH518-2	79	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
359	BH518-4	79	0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
368	deisopropylatrazine	5	0.44	0.19	8.83	3.90	<0.01	<0.01	<0.01	<0.01
416	methomyl	2.5	0.97	0.40	**38.66**	**16.16**	<0.01	<0.01	<0.01	<0.01
442	diisopropylamine	25	0.01	<0.01	0.05	0.01	<0.01	<0.01	<0.01	<0.01
470	2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	15	<0.01	<0.01	0.02	0.01	<0.01	<0.01	<0.01	<0.01

Table A11.3. Total indirect adult exposure to pesticide metabolites for Catchment B

Metabolite code	Pesticide Metabolite	ADI[1] (µg/kg bw/day)	Total Daily Intake (µg/kg bw/day) Conservative Estimate		%ADI Conservative Estimate		Total Daily Intake (µg/kg bw/day) Refined Estimate II		%ADI Refined Estimate II	
			Conventional	Advanced	Conventional	Advanced	Conventional	Advanced	Conventional	Advanced
3	cis-3-chloroprop-2-enoic acid	12.5	0.16	0.07	1.30	0.57	<0.01	<0.01	<0.01	<0.01
5	trans-3-chloroprop-2-enoic acid	12.5	0.16	0.07	1.30	0.57	<0.01	<0.01	<0.01	<0.01
14	aldicarb sulfone	1	0.24	0.08	**23.71**	7.83	<0.01	<0.01	<0.01	<0.01
15	aldicarb sulfoxide	1.25	0.01	<0.01	0.74	0.22	<0.01	<0.01	<0.01	<0.01
25	sulfanilamide	360	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
26	deethylatrazine	20	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
33	reference compound 10	100	0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
61	2-aminobenzimidazole	20	0.06	0.01	0.30	0.07	<0.01	<0.01	<0.01	<0.01
63	carbofuran	1	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
65	carboxin sulfoxide	1.6	0.02	<0.01	1.14	0.11	<0.01	<0.01	<0.01	<0.01
67	5-amino-4-chloropyridazin-3(2H)-one	100	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
69	3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	18	0.47	0.47	2.62	2.62	<0.01	<0.01	<0.01	<0.01
70	3-carbamyl-2,4,5-trichlorobenzoic acid	500	0.13	0.13	0.03	0.03	<0.01	<0.01	<0.01	<0.01
73	3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	5	0.01	<0.01	0.17	0.07	<0.01	<0.01	<0.01	<0.01
75	R417888	60	0.62	0.25	1.03	0.42	<0.01	<0.01	<0.01	<0.01
76	3-(3-chloro-p-tolyl)-1-methylurea	40	0.04	0.01	0.11	0.02	<0.01	<0.01	<0.01	<0.01
84	cyanazine acid	2	0.09	0.04	4.48	1.99	<0.01	<0.01	<0.01	<0.01
114	AE 0542291	200	0.02	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
166	5-hydroxy-XDE-570	50	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
169	5-trifluoromethyl-pryid-2-one	10	0.03	0.01	0.30	0.11	<0.01	<0.01	<0.01	<0.01
174	FOE oxalte	4	0.02	0.01	0.41	0.17	<0.01	<0.01	0.02	0.01
176	thiadone	4	0.01	0.01	0.32	0.16	<0.01	<0.01	0.01	0.01
190	ethanol	24	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
191	phosphorous acid	3900	0.11	0.11	<0.01	<0.01	0.01	0.01	<0.01	<0.01
199	aminomethylphosphonic acid	300	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
205	1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	60	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
210	AE F145740	30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
211	metsulfuron-methyl	250	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
212	3,5-di-iodo-4-hydroxybenzamide	50	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Metabolite code	Pesticide Metabolite	ADI[1] (µg/kg bw/day)	Total Daily Intake (µg/kg bw/day) Conservative Estimate		%ADI Conservative Estimate		Total Daily Intake (µg/kg bw/day) Refined Estimate II		%ADI Refined Estimate II	
213	3,5-di-iodo-4-hydroxybenzoic acid	50	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
217	3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	15	<0.01	<0.01	0.03	0.01	<0.01	<0.01	0.01	<0.01
218	desmethylisoproturon	15	0.01	<0.01	0.03	0.01	<0.01	<0.01	0.01	<0.01
255	acetaldehyde	20	0.22	0.22	1.11	1.11	<0.01	<0.01	<0.01	<0.01
258	metazachlor oxalic acid	80	0.02	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01
259	metazachlor sulfonic acid	80	0.08	<0.01	0.11	<0.01	0.01	<0.01	0.01	<0.01
266	methiocarb sulfoxide	13	0.04	0.01	0.30	0.05	<0.01	<0.01	<0.01	<0.01
270	ATSA	5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
271	demethyl metoxuron	740	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
276	diketo metribuzin	13	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
279	IN-D5119	250	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
280	IN-D5803	250	0.02	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
295	dimethyloxamic acid	1	0.02	0.01	1.94	0.80	<0.01	<0.01	0.26	0.11
299	2,6-dinitro-3,4-xylidine	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
300	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
301	4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
304	4-fluoroaniline	14	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
349	CGA 294849	30	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
358	BH518-2	79	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
359	BH518-4	79	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
368	deisopropylatrazine	5	0.17	0.08	3.44	1.52	<0.01	<0.01	<0.01	<0.01
416	methomyl	2.5	0.42	0.18	**16.75**	7.00	<0.01	<0.01	<0.01	<0.01
442	diisopropylamine	25	0.01	<0.01	0.02	0.01	<0.01	<0.01	<0.01	<0.01
470	2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table A11.4. Total indirect toddler exposure to pesticide metabolites for Catchment B

Metabolite code	Pesticide Metabolite	ADI[1] (µg/kg bw/day)	Total Daily Intake (µg/kg bw/day) Conservative Estimate		%ADI Conservative Estimate		Total Daily Intake (µg/kg bw/day) Refined Estimate II		%ADI Refined Estimate II	
			Conventional	Advanced	Conventional	Advanced	Conventional	Advanced	Conventional	Advanced
3	cis-3-chloroprop-2-enoic acid	12.5	0.73	0.33	5.87	2.60	<0.01	<0.01	<0.01	<0.01
5	trans-3-chloroprop-2-enoic acid	12.5	0.73	0.33	5.87	2.60	<0.01	<0.01	<0.01	<0.01
14	aldicarb sulfone	1	1.07	0.35	**107.41**	**35.45**	<0.01	<0.01	<0.01	<0.01
15	aldicarb sulfoxide	1.25	0.04	0.01	3.34	0.98	<0.01	<0.01	<0.01	<0.01
25	sulfanilamide	360	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
26	deethylatrazine	20	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01
33	reference compound 10	100	0.03	0.01	0.03	0.01	<0.01	<0.01	<0.01	<0.01
61	2-aminobenzimidazole	20	0.27	0.06	1.34	0.30	<0.01	<0.01	<0.01	<0.01
63	carbofuran	1	<0.01	<0.01	0.03	0.01	<0.01	<0.01	<0.01	<0.01
65	carboxin sulfoxide	1.6	0.08	0.01	5.17	0.52	<0.01	<0.01	<0.01	<0.01
67	5-amino-4-chloropyridazin-3(2H)-one	100	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
69	3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	18	2.13	2.13	**11.86**	**11.86**	<0.01	<0.01	0.01	0.01
70	3-carbamyl-2,4,5-trichlorobenzoic acid	500	0.60	0.60	0.12	0.12	<0.01	<0.01	<0.01	<0.01
73	3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	5	0.04	0.02	0.77	0.30	<0.01	<0.01	<0.01	<0.01
75	R417888	60	2.81	1.13	4.68	1.88	<0.01	<0.01	0.01	<0.01
76	3-(3-chloro-p-tolyl)-1-methylurea	40	0.19	0.04	0.48	0.10	<0.01	<0.01	<0.01	<0.01
84	cyanazine acid	2	0.41	0.18	**20.31**	9.02	<0.01	<0.01	<0.01	<0.01
114	AE 0542291	200	0.08	0.02	0.04	0.01	<0.01	<0.01	<0.01	<0.01
166	5-hydroxy-XDE-570	50	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
169	5-trifluoromethyl-pryid-2-one	10	0.14	0.05	1.37	0.48	<0.01	<0.01	<0.01	<0.01
174	FOE oxalte	4	0.07	0.03	1.86	0.76	<0.01	<0.01	0.07	0.03
176	thiadone	4	0.06	0.03	1.46	0.73	<0.01	<0.01	0.05	0.03
190	ethanol	24	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
191	phosphorous acid	3900	0.49	0.49	0.01	0.01	0.03	0.03	<0.01	<0.01
199	aminomethylphosphonic acid	300	0.04	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
205	1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	60	0.02	0.01	0.04	0.01	<0.01	<0.01	<0.01	<0.01
210	AE F145740	30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
211	metsulfuron-methyl	250	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
212	3,5-di-iodo-4-hydroxybenzamide	50	0.01	<0.01	0.02	0.01	<0.01	<0.01	<0.01	<0.01

Metabolite code	Pesticide Metabolite	ADI[1] (µg/kg bw/day)	Total Daily Intake (µg/kg bw/day) Conservative Estimate		%ADI Conservative Estimate		Total Daily Intake (µg/kg bw/day) Refined Estimate II		%ADI Refined Estimate II	
213	3,5-di-iodo-4-hydroxybenzoic acid	50	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
217	3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	15	0.02	<0.01	0.15	0.03	0.01	<0.01	0.04	0.01
218	desmethylisoproturon	15	0.02	<0.01	0.15	0.03	0.01	<0.01	0.04	0.01
255	acetaldehyde	20	1.01	1.01	5.05	5.05	<0.01	<0.01	0.02	0.02
258	metazachlor oxalic acid	80	0.10	0.01	0.13	0.02	0.01	<0.01	0.01	<0.01
259	metazachlor sulfonic acid	80	0.38	<0.01	0.48	<0.01	0.03	<0.01	0.04	<0.01
266	methiocarb sulfoxide	13	0.18	0.03	1.38	0.21	<0.01	<0.01	0.02	<0.01
270	ATSA	5	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
271	demethyl metoxuron	740	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
276	diketo metribuzin	13	0.01	<0.01	0.04	0.02	<0.01	<0.01	<0.01	<0.01
279	IN-D5119	250	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
280	IN-D5803	250	0.08	0.02	0.03	0.01	<0.01	<0.01	<0.01	<0.01
295	dimethyloxamic acid	1	0.09	0.04	8.77	3.63	0.01	<0.01	1.18	0.49
299	2,6-dinitro-3,4-xylidine	125	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
300	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
301	4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
304	4-fluoroaniline	14	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
349	CGA 294849	30	0.02	0.01	0.06	0.02	<0.01	<0.01	<0.01	<0.01
358	BH518-2	79	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
359	BH518-4	79	0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
368	deisopropylatrazine	5	0.78	0.34	**15.60**	6.88	<0.01	<0.01	<0.01	<0.01
416	methomyl	2.5	1.90	0.79	**75.86**	**31.71**	<0.01	<0.01	<0.01	<0.01
442	diisopropylamine	25	0.02	0.01	0.10	0.03	<0.01	<0.01	<0.01	<0.01
470	2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	15	<0.01	<0.01	0.02	0.01	<0.01	<0.01	<0.01	<0.01

Table A11.5. Total indirect adult exposure to pesticide metabolites for Catchment C

Metabolite code	Pesticide Metabolite	ADI[1] (µg/kg bw/day)	Total Daily Intake (µg/kg bw/day) Conservative Estimate		%ADI Conservative Estimate		Total Daily Intake (µg/kg bw/day) Refined Estimate II		%ADI Refined Estimate II	
			Conventional	Advanced	Conventional	Advanced	Conventional	Advanced	Conventional	Advanced
3	cis-3-chloroprop-2-enoic acid	12.5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
5	trans-3-chloroprop-2-enoic acid	12.5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
14	aldicarb sulfone	1	<0.01	<0.01	0.28	0.09	<0.01	<0.01	<0.01	<0.01
15	aldicarb sulfoxide	1.25	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
25	sulfanilamide	360	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
26	deethylatrazine	20	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
33	reference compound 10	100	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
61	2-aminobenzimidazole	20	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
63	carbofuran	1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
65	carboxin sulfoxide	1.6	<0.01	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01
67	5-amino-4-chloropyridazin-3(2H)-one	100	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
69	3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	18	0.01	0.01	0.05	0.05	<0.01	<0.01	<0.01	<0.01
70	3-carbamyl-2,4,5-trichlorobenzoic acid	500	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
73	3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
75	R417888	60	0.01	<0.01	0.02	0.01	<0.01	<0.01	<0.01	<0.01
76	3-(3-chloro-p-tolyl)-1-methylurea	40	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
84	cyanazine acid	2	<0.01	<0.01	0.04	0.02	<0.01	<0.01	<0.01	<0.01
114	AE 0542291	200	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
166	5-hydroxy-XDE-570	50	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
169	5-trifluoromethyl-pryid-2-one	10	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
174	FOE oxalte	4	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
176	thiadone	4	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
190	ethanol	24	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
191	phosphorous acid	3900	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
199	aminomethylphosphonic acid	300	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
205	1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	60	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
210	AE F145740	30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
211	metsulfuron-methyl	250	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
212	3,5-di-iodo-4-hydroxybenzamide	50	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Metabolite code	Pesticide Metabolite	ADI[1] (µg/kg bw/day)	Total Daily Intake (µg/kg bw/day) Conservative Estimate		%ADI Conservative Estimate		Total Daily Intake (µg/kg bw/day) Refined Estimate II		%ADI Refined Estimate II	
213	3,5-di-iodo-4-hydroxybenzoic acid	50	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
217	3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
218	desmethylisoproturon	15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
255	acetaldehyde	20	<0.01	<0.01	0.02	0.02	<0.01	<0.01	<0.01	<0.01
258	metazachlor oxalic acid	80	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
259	metazachlor sulfonic acid	80	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
266	methiocarb sulfoxide	13	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
270	ATSA	5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
271	demethyl metoxuron	740	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
276	diketo metribuzin	13	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
279	IN-D5119	250	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
280	IN-D5803	250	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
295	dimethyloxamic acid	1	<0.01	<0.01	0.05	0.02	<0.01	<0.01	<0.01	<0.01
299	2,6-dinitro-3,4-xylidine	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
300	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
301	4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
304	4-fluoroaniline	14	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
349	CGA 294849	30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
358	BH518-2	79	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
359	BH518-4	79	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
368	deisopropylatrazine	5	<0.01	<0.01	0.06	0.03	<0.01	<0.01	<0.01	<0.01
416	methomyl	2.5	0.01	<0.01	0.36	0.15	<0.01	<0.01	<0.01	<0.01
442	diisopropylamine	25	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
470	2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table A11.5. Total indirect toddler exposure to pesticide metabolites for Catchment C

Metabolite code	Pesticide Metabolite	ADI[1] (µg/kg bw/day)	Total Daily Intake (µg/kg bw/day) Conservative Estimate		%ADI Conservative Estimate		Total Daily Intake (µg/kg bw/day) Refined Estimate II		%ADI Refined Estimate II	
			Conventional	Advanced	Conventional	Advanced	Conventional	Advanced	Conventional	Advanced
3	cis-3-chloroprop-2-enoic acid	12.5	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
5	trans-3-chloroprop-2-enoic acid	12.5	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
14	aldicarb sulfone	1	0.01	<0.01	1.27	0.42	<0.01	<0.01	<0.01	<0.01
15	aldicarb sulfoxide	1.25	<0.01	<0.01	0.04	0.01	<0.01	<0.01	<0.01	<0.01
25	sulfanilamide	360	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
26	deethylatrazine	20	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
33	reference compound 10	100	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
61	2-aminobenzimidazole	20	<0.01	<0.01	0.02	0.01	<0.01	<0.01	<0.01	<0.01
63	carbofuran	1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
65	carboxin sulfoxide	1.6	<0.01	<0.01	0.11	0.01	<0.01	<0.01	<0.01	<0.01
67	5-amino-4-chloropyridazin-3(2H)-one	100	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
69	3-carbamyl-1,2,4,5-tetrachlorobenzoic acid	18	0.04	0.04	0.22	0.22	<0.01	<0.01	<0.01	<0.01
70	3-carbamyl-2,4,5-trichlorobenzoic acid	500	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
73	3-cyano-6-hydroxy-2,4,5-trichlorobenzamide	5	<0.01	<0.01	0.01	0.01	<0.01	<0.01	<0.01	<0.01
75	R417888	60	0.04	0.02	0.07	0.03	<0.01	<0.01	<0.01	<0.01
76	3-(3-chloro-p-tolyl)-1-methylurea	40	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
84	cyanazine acid	2	<0.01	<0.01	0.20	0.09	<0.01	<0.01	<0.01	<0.01
114	AE 0542291	200	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
166	5-hydroxy-XDE-570	50	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
169	5-trifluoromethyl-pryid-2-one	10	<0.01	<0.01	0.03	0.01	<0.01	<0.01	<0.01	<0.01
174	FOE oxalte	4	<0.01	<0.01	0.04	0.01	<0.01	<0.01	<0.01	<0.01
176	thiadone	4	<0.01	<0.01	0.03	0.01	<0.01	<0.01	<0.01	<0.01
190	ethanol	24	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
191	phosphorous acid	3900	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
199	aminomethylphosphonic acid	300	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
205	1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine	60	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
210	AE F145740	30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
211	metsulfuron-methyl	250	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
212	3,5-di-iodo-4-hydroxybenzamide	50	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Metabolite code	Pesticide Metabolite	ADI[1] (µg/kg bw/day)	Total Daily Intake (µg/kg bw/day) Conservative Estimate		%ADI Conservative Estimate		Total Daily Intake (µg/kg bw/day) Refined Estimate II		%ADI Refined Estimate II	
213	3,5-di-iodo-4-hydroxybenzoic acid	50	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
217	3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea	15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
218	desmethylisoproturon	15	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
255	acetaldehyde	20	0.02	0.02	0.09	0.09	<0.01	<0.01	<0.01	<0.01
258	metazachlor oxalic acid	80	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
259	metazachlor sulfonic acid	80	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
266	methiocarb sulfoxide	13	<0.01	<0.01	0.03	<0.01	<0.01	<0.01	<0.01	<0.01
270	ATSA	5	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
271	demethyl metoxuron	740	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
276	diketo metribuzin	13	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
279	IN-D5119	250	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
280	IN-D5803	250	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
295	dimethyloxamic acid	1	<0.01	<0.01	0.21	0.09	<0.01	<0.01	0.01	<0.01
299	2,6-dinitro-3,4-xylidine	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
300	4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
301	4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid	125	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
304	4-fluoroaniline	14	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
349	CGA 294849	30	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
358	BH518-2	79	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
359	BH518-4	79	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
368	deisopropylatrazine	5	0.01	0.01	0.26	0.11	<0.01	<0.01	<0.01	<0.01
416	methomyl	2.5	0.04	0.02	1.61	0.67	<0.01	<0.01	<0.01	<0.01
442	diisopropylamine	25	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
470	2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole	15	<0.01	<0.01	0.02	0.01	<0.01	<0.01	<0.01	<0.01

Appendix 12 – Hazard assessments of selected metabolites

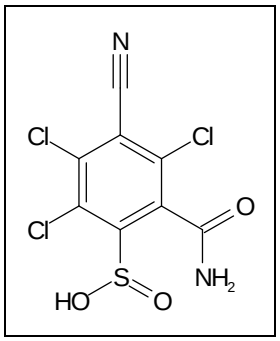
Appendix 12.1 R417888 (2-Amido-3,5,6-trichloro-4-cyanobenzenesulphonic acid)

Introduction

R417888 is a major metabolite of the fungicide chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile; CAS No. 1897-45-6) formed within the surrounding soil. It is also formed through metabolism of absorbed chlorothalonil in humans.

The structure of R417888, is presented in Figure 1.

Figure 1: Structure of R417888



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of chlorothalonil

Chlorothalonil is a broad-spectrum non-systemic pesticide that is used primarily as a fungicide and mildewicide but has some additional activity as a bactericide, microbiocide, algaecide, insecticide and acaricide. Chlorothalonil was first registered in the US in 1966 for application to turf but is now registered for a wide range of applications including on fields, vegetables and orchard crops and as a mildewicide in paint and other surface treatments (US EPA, 1999).

Chlorothalonil is currently sold worldwide under several commercial names including Bravo, Daconil 2787, Echo, Exotherm Termil, Forturf, Mold-Ex, Nopcocide N-96, Ole, Pillarich, Repulse and Tuffcide. In general it is sold as a soluble concentrate that is mixed with water and applied as a spray. Application rates vary depending on use. For most crops the maximum rate of application is between 0.28 – 0.41 kg chlorothalonil per hectare although two crops require a higher rate of 0.83-0.92 kg chlorothalonil per hectare (ENVIRO*facts*, 2003).

2.2 Environmental fate

2.2.1 Parent

Any chlorothalonil released into the air during application is likely to enter both vapour and particulate phases. Chlorothalonil within the vapour phase will be slowly photochemically degraded with an estimated half-life of 7 years (HSDB, 2006).

Although photolysis might also occur in the particulate phase, no data on the rate at which this might occur is available. Particulate-phase chlorothalonil is mainly removed from the atmosphere by wet and dry deposition.

Chlorothalonil has low water solubility (0.81 mg/L at 25^{o}C, pH (neutral); EC, 2006). K_{oc} values of between 900 and 7000 L kg^{-1} have been described (HSDB, 2006) suggesting slight or no mobility to soils.

Chlorothalonil is moderately susceptible to degradation in soil under aerobic conditions (US EPA, 1999). Degradation occurs mainly through dechlorination and some substitution reactions (Sato et al., 1987). The degradation half-life in four soils has been reported to be between 10 and 40 days (HSDB, 2006); the average half-life was 22 days (Lewis *et al*., 2007). Chlorothalonil is not considered to be persistent.

2.2.2 Metabolite: R417888

R417888 is one of the major metabolites formed from chlorothalonil, with an estimated maximum formation fraction of 0.200 (20%; Lewis *et al*., 2007).

A K_{oc} value of 10 L kg^{-1} has been reported for R417888 (Lewis *et al*., 2007) suggesting high mobility in the environment.

The degradation half-life of R417888 in soil under aerobic conditions is estimated to be 121.1 days and, hence, it is considered to be persistent (Lewis *et al.,* 2007).
No further information on the physicochemical properties or environmental fate of R417888 was identified.

2.3 Potential routes of human exposure

During production chlorothalonil may be released to the environment through various waste streams. Direct release into the environment will also occur during its use as a pesticide. Exposure to chlorothalonil may occur through ingestion of contaminated food and water or through inhalation or dermal contact during occupational handling or by-stander exposure (HSDB, 2006).

Although no data on routes of exposure of humans to the metabolite R417888 was identified, it is plausible that exposure may occur as a result of its ingestion in food or water contaminated from the breakdown of chlorothalonil, or when formed as a human metabolite from ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of chlorothalonil in humans.

Toxicokinetic studies have been carried in Sprague-Dawley rats orally dosed with ^{14}[C]-chlorothalonil at 5, 50 or 200 mg/kg. In males, approximately 89% of the radioactive dose was excreted and in females approximately 96%. The major route of excretion was the faeces (83 - 87%) with excretion apparently complete within 48 hr in low dose females and low and mid dose males, and by 72 hr in mid/high dose females and high dose males. Some urinary excretion also occurred which showed saturation at higher doses. At low doses, urinary excretion was 92 - 93% complete within 24 hr, and for mid doses within 48 hr, however, at high doses only 95% excretion was achieved by 72 hr (IPCS, 1996).

A similar pattern was noted in rats given a single low oral dose (1.5mg/kg) of chlorothalonil. Around 30 - 32% of administered dose was absorbed from the GI tract, with approximately 20 - 22% of the absorbed dose being excreted in bile, and a further 10% excreted in urine (Krieger, 2001).

In monkeys treated dermally with 5 mg/kg bw of ^{14}[C]-chlorothalonil under a non-occlusive patch for 48 hr, around 90% of the dose was recovered from the treated surface, i.e. unabsorbed; only 2.26% was absorbed through the skin. Urine contained 1% of the absorbed dose but this did not include any detectable methylated mono-, di- and trithiols (IPCS, 1996). In male Sprague-Dawley rats given 200 mg/kg ^{14}[C]-chlorothalonil by oral gavage, urine collected at 17, 24 and 48 hr following

administration contained the metabolites trimethylthiomonochloro-isophthalonitrile and dimethylthiodichloro-isophthalonitrile, excreted as free thiols or methylated derivatives, and accounted for 2.4% of the administered dose (IPCS, 1996).

In a repeat oral dose study male rats were given ^{14}[C]-chlorothalonil at 1.5, 5, 50 or 160 mg/kg/day, and culled 2, 9, 24, 96 and 168 hr following administration of the last dose. Chlorothalonil was found to be widely distributed, with highest concentrations in the kidneys followed by liver and blood. Peak concentrations were seen 2 hr after the last dose, at which time 0.28% and 0.20% of the radioactivity was found in the kidney for the 1.5 and 5 mg/kg dose levels respectively (IPCS, 1996).

In a further study, rats were given repeated doses of ^{14}[C]-chlorothalonil at up to 160 mg/kg/day. Methylated or partly methylated dithiol and trithiol derivatives were identified in urine at all doses (IPCS, 1996).

No information on the toxicokinetics of R417888 was identified.

4 Toxicity Profile

4.1 Chlorothalonil

4.1.1 Acute Toxicity

No information is available on the acute toxicity of chlorothalonil to humans.

Chlorothalonil has low acute toxicity in rats; LD_{50}'s are 5000 mg/kg, 2000 mg/kg and 0.1 mg/m^3 when given by oral, dermal or inhalation routes respectively (EC, 2006).

Chlorothalonil is a contact irritant to the skin, eye and respiratory tract (US EPA, 1999) and there is evidence to suggest that it is a skin sensitiser (EC, 2006).

4.1.2 Repeat dose toxicity

Repeated exposure of humans to chlorothalonil has been associated with development of contact dermatitis and other skin conditions. For example, employees in a chlorothalonil manufacturing plant were reported to have an increased incidence (60%) of skin abnormalities compared with non-exposed workers (18.5%; IPCS, 1996).

In animal studies, the principal target organs of repeated exposure are considered to be the kidney and forestomach.

In a 2 year feeding study, Fischer-344 rats were administered chlorothalonil via the diet at up to 175mg/kg bw/day. Histopathological examination of the kidneys showed nephropathy, tubular hyperplasia (considered a preneoplastic change) and chronic progressive nephropathy in rats of treated groups. Increased incidence and severity of hyperplasia and hyperkeratosis of the squamous mucosa of the oesophagus and forestomach were also noted in treated groups; the stomach and oesophageal changes were attributed to an irritant effect of chlorothalonil. A NOEL of 1.8 mg/kg bw/day was established for non-neoplastic effects (IPCS, 1996).

Similar findings were noted in Beagle dogs administered chlorothalonil at dietary levels up to 750 mg/kg bw/day for 2 years, with treatment-related histopathological changes being seen in the liver, thyroid, kidney and stomach at mid and high doses (IPCS, 1996).

4.1.3 Carcinogenicity and mutagenicity

There is inadequate evidence of carcinogenicity of chlorothalonil in humans. However, IARC has noted that there is *sufficient evidence of carcinogenicity in experimental animals*, and concluded that chlorothalonil is *possibly carcinogenic to humans* (Group 2B; IARC, 1999).

In the two year dietary study in which Fischer-344 rats were administered chlorothalonil at up to 175 mg/kg bw/day described above, the incidence of renal tubular adenoma and carcinoma was increased in treated male and female groups. A treatment related increase in incidence and severity of renal tubular epithelial hyperplasia was also noted and was considered a pre-neoplastic change. Hyperplasia and hyperkeratosis of the squamous mucosa of the oesophagus and forestomach were also apparent (IPCS, 1996).

The carcinogenic potential of chlorothalonil administered in the diet at doses up to 165 mg/kg bw/day was assessed in a further rat study; a NOEL of 3.8 mg/kg bw/day was established (IPCS, 1996).

Evidence on the genotoxic potential of chlorothalonil is conflicting. Although some *in vitro* models without activation have shown positive results, the available *in vivo* studies were inconclusive (EC, 2006; HSDB, 2006).

4.1.4 Reproductive and development toxicity
No information on the reproductive and developmental toxicity of chlorothalonil in humans was identified.

In a two-generation reproduction study in Charles River CD rats, chlorothalonil was administered via the diet at up to 3000 mg/kg diet. No treatment-related effects were noted in F_0 or F_1 parent animals and reproductive parameters were unaffected. However pups displayed lower bodyweights. A NOEL for this effect of 75 mg/kg bw/day was proposed (IPCS, 1996).

Similar results have been described in rabbits when given chlorothalonil by oral gavage at up to 20 mg/kg/day on days 7 - 19 of gestation. A lowered maternal body weight in conjunction with decreased food consumption was noted at the highest dose; pregnancy rate was $\geq$ 95% in all groups and no evidence of fetotoxicity or teratogenicity was found (IPCS, 1996).

4.2 R417888
4.2.1 Acute Toxicity
No information on the acute toxic effects of R417888 in humans was identified.
In rats, R417888 shows moderate acute toxicity, with an oral LD_{50} of 2000 mg/kg (EC, 2006).

4.2.2 Repeat dose toxicity
No information on the repeat dose toxicity of R417888 in humans was identified.

Experimentally, the principal target organ for R417888 toxicity is the kidney, with a reported NOAEL of > 59 mg/kg bw/day being given following a 90 day dietary study in rats (EU, 2006; from non-published, data-protected company reports).

Further searches were made for publicly available information on the toxicity of R417888, including use of the online programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1: Predicted toxicity data for R417888 using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H(range)				
Chlorothalonil (experimental data)	5000	0.1	NOEL: 1.8 mg/kg/day. Main target organs: kidney and fore-stomach	Genotoxic potential not established	Fore-stomach tumours in rats and mice; kidney tumours in mice	Reduced pup weight (rats).
R417888 (experimental data)	> 2000	n/d	NOAEL: >59 mg/kg bw/day. Main target organ: kidney	n/d	n/d	n/d
R417888 (TOPKAT)	227.9 (48.2-1100)	518.2 (56.7-4200)	MTD (feed/drink) 1300 mg/kg MTD (oral - gavage) 1300 mg/kg	Negative	Negative	Predicted developmental toxicant
R417888 (DEREK)	n/a	n/a	*Improbable* that R417888 will cause photoallergen-icity or skin sensitisation	No alert	*Equivocal*	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software

DEREK identified the presence of only one structural alert and the following conclusion was drawn:

It is considered improbable (i.e. there is little supporting evidence) that R417888 will cause photoallergenicity or skin sensitisation in humans. This endpoint is predicted because R417888 is a polyhalogenated aromatic compound.

The analysis by TOPKAT suggested that R417888 was:

non-carcinogenic;
non-mutagenic; but
a developmental toxicant.

5 Guidelines and Standards
5.1 Chlorothalonil

The EC risk classification is: Carcinogen category 3: R40; T+ - very toxic: R26; Xi – Irritant: R37, R41, R43; N – Dangerous for the environment: R50, R53. Chlorothalonil is classified by WHO as 'unlikely to present acute hazard in normal use' and by the US EPA as 'moderately toxic' (Lewis *et al*., 2007).

An ADI of 0.018 mg/kg bw/d has been established for chlorothalonil using a safety factor (SF) of 100, based on a two year rat study in which Fischer 344 rats received chlorothalonil via the diet at up to 175 mg/kg/day (IPCS, 1996; Lewis *et al*., 2007).

5.2 R417888

R417888 is not listed under WHO, US EPA or EC classifications.

An ADI of 0.06 mg/kg bw/day has been proposed for R417888, based on a NOAEL of > 59 mg/kg, determined from a 90 day toxicity study in rats, with a SF of 1000 applied (EC, 2006; Lewis *et al.*, 2007). In view of the low concern identified by the predictive software systems, this value will be adopted in the risk assessment.

6 References

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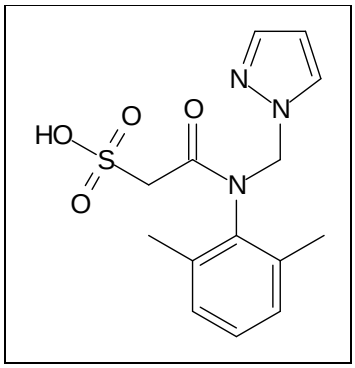
Appendix 12.2 Metazachlor sulfonic acid (479M08)

1 Introduction

Metazachlor sulfonic acid is a major metabolite of the herbicide metazachlor (2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(1*H*-pyrazol-1-ylmethyl) acetamide); CAS No. 67129-08-2). It is formed within the plant, surrounding soil and in groundwater. This metabolite is also formed during the metabolism of absorbed metazachlor by humans.

The structure of metazachlor sulfonic acid is presented in Figure 1.

Figure 1: Structure of metazachlor sulfonic acid



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Metazachlor

Metazachlor is used as a selective herbicide for the control of annual grasses and broad-leaved weeds in a wide range of crops (artichokes, broccoli, asparagus, Brussels sprouts, cabbages, cauliflowers, sweet corn, garlic, horseradish, kale, leeks, maize, white mustard, onions, peanuts, pome fruits, potatoes, radish, rape, soya beans, stone fruits, strawberries, sugar cane, sunflowers, tobacco and turnips; Pesticide Manual, 1997).

Metazachlor is currently registered for use in around 40 countries in the EU, Africa and Asia and is marketed under several commercial names including Alpha Metazachlor, Nimbus CS, Rapasan 500SC, Shadow, Springbok and Sultan. In general, it is formulated as a suspension concentrate and applied as a spray (Lewis *et al*., 2007).

2.2 Environmental fate

2.2.1 Parent

Metazachlor is moderately soluble in water (450 mg/L at 20oC, Lewis et al., 2006). In assessing the sorption to soil, a K_{oc} value of 134 L kg^{-1} has been described (Lewis *et al*., 2007) suggesting moderate environmental mobility.

The volatility of metazachlor is low and therefore losses to the atmosphere from plant surfaces are expected to be small. Degradation of metazachlor in the atmosphere is relatively rapid; atmospheric degradation half-life is around 6.5 hr (EFSA, 2008) and it is therefore not considered to be persistent in the atmosphere.

Metazachlor is relatively rapidly degraded in soil with mean degradation half-lives of 10.8 days (at 20ºC) under laboratory conditions and 6.8 days under field conditions (EFSA, 2008).

2.2.2 Metabolite: metazachlor sulfonic acid

Metazachlor sulfonic acid is one of the major metabolites formed during the metabolism or degradation of metazachlor, with an estimated formation fraction of 0.216 (approximately 20%; Lewis *et al*., 2007).

A K_{oc} value of 41 L kg^{-1} has been reported for metazachlor sulfonic acid (Lewis *et al*., 2007) suggesting that the metabolite is mobile in the environment.

The degradation half-life of metazachlor sulfonic acid in soil under laboratory conditions is 123.2 days. Under field conditions, the geometric mean half-life at 20ºC was 71.1 days (EFSA, 2008) and the metabolite is considered persistent (Lewis *et al.,* 2007).

Metazachlor sulfonic acid is predicted to have high leachability, with a GUS leaching potential index of 3.53 and the SCI-GROW groundwater index (µg l^{-1} for a 1 kg ha^{-1} or 1 l ha^{-1} application) has been calculated as 6.51 X 10^{-01}µg L^{-1}(Lewis et al., 2007).
No further information relating to either the physicochemical properties or environmental fate of metazachlor sulfonic acid was identified.

2.3 Potential routes of human exposure

Exposure to metazachlor may occur through ingestion of contaminated food and water or by inhalation or dermal contact through occupational handling or bystander exposure (EFSA, 2008).

Although no data on routes of exposure of humans to the metabolite metazachlor sulfonic acid were identified, it is plausible that exposure may occur as a result of its ingestion in food or water contaminated from the breakdown of metazachlor, or when formed as a human metabolite from ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of metazacholor in humans.

Experimentally, CD rats were administered ^{14}[C]-metazachlor at 300 mg/kg bw by oral gavage as a single dose or on each of seven consecutive days. Following administration of the single dose, absorption was rapid with plasma radioactivity detectable within 15 min, and maximum levels being reached by 8 hr, for both sexes. However, radioactivity levels were approximately twice as high in females compared with males. Metazachlor was rapidly and widely distributed, with extensive metabolism occurring by a number of routes including hydroxylation of the pyrazole group, oxidation of the methylphenyl group, oxidative dechlorination, and glutathione and glucuronide conjugation. Excretion was predominantly via the urine (around 60 – 70%) in both sexes but was more rapid in females.

In the repeat dose study, six hours following administration of the final dose of ^{14}[C]-metazachlor, the highest level of radioactivity was associated with the GI tract. High levels were also maintained in whole blood; however, levels in plasma were much lower and showed a fall with time suggesting that binding of metazachlor or its metabolites might be occurring. Binding to cellular components was attributed mainly to the liver and kidney. Although excretion was essentially complete within 144hr following administration of the final dose, relatively high levels of radioactivity were detected in the spleen, thyroid and lungs up to 10 days later (Hawkins *et al.,* 1980).
No information on the toxicokinetics of metazachlor sulfonic acid was identified.

4 Toxicity Profile

4.1 Metazachlor

4.1.1 Acute Toxicity

No information is available on the acute toxicity of metazachlor in humans.

Experimentally, metazachlor has moderate acute toxicity in rats; LD_{50}'s are 2000 mg/kg, 2000 mg/kg and 34.5 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis *et al*., 2007).

Metazachlor is not a skin or eye irritant (EFSA, 2008) but there is evidence to suggest that it is a skin sensitiser (Gelbke & Grundler, 1980).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of metazachlor in humans was identified.
Experimentally, the principal target organ of metazachlor toxicity is the liver. Increased liver weights and enlarged, vacuolated hepatocytes were noted in male and female Sprague-Dawley rats given metazachlor via the diet at up to 6000 ppm for up to 113 weeks. At high doses, changes in clinical chemistry parameters were consistent with hepatotoxicity and haemolytic anaemia was also noted. No NOAEL was proposed (Hunter, 1983).

In a further study, Wistar rats were administered metazachlor in the diet at up to 8000 ppm for 2 years; bodyweight gain and food consumption were significantly reduced in males at 2000 ppm or above, and in females at 8000 ppm. In both sexes, clinical chemistry changes at high doses indicated a mild macrocytic anaemia, whilst at doses ≥ 200 ppm, an increase in total plasma bilirubin was evident. Mean and absolute liver and kidney weights were also increased at the highest dose. A NOAEL of 200 ppm (equivalent to 8.5 and 11.6 mg/kg bw/d in males and females respectively) was proposed based on a reduction in body weight gain and food consumption, and elevated plasma bilirubin levels (Krishnappa, 2002).

4.1.3 Carcinogenicity and mutagenicity

No information on the carcinogenicity and mutagenicity of metazachlor in humans was identified.

In the 2 year rat feeding study described above (Hunter, 1983), an increase in the incidence of thyroid parafollicular adenoma and thyroid parafollicular carcinoma was noted in male rats administered doses of 2000 or 6000 ppm metazachlor. Although a slightly higher incidence of hepatic adenoma were noted in male rats given 6000 ppm, this was not considered relevant to human risk assessment as this dose was also associated with hepatotoxicity in the rats (Hunter, 1983).

Metazachlor has been tested in a number of *in vitro* and *in vivo* genotoxicity studies. However, it has been concluded that metazachlor does not have genotoxic potential (EFSA, 2008).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of metazachlor in humans was identified.

In a two-generation reproduction study in which Crl:COBS CD(SD) rats were administered metazachlor via the diet at up to 1000 ppm for 15 weeks, no evidence reproductive toxicity was seen (Cozens, 1982). Parental and reproductive NOAELs of 1000 ppm were proposed (EFSA, 2008).

In a three-generation study in Wistar rats fed treated diet containing metazachlor at up to 8000 ppm, reduced litter sizes (secondary to reduced numbers of corpora lutea and implantations), together with reduced pup weight and post-natal survival, were seen at the high dose (Ganiger 1999). Parental and reproductive NOAELs of 151mg/kg bw/day and 192 mg/kg bw/day (2000 ppm) and a developmental NAOEL of 200 ppm (equivalent to 20 mg/kg bw/day) were proposed (EFSA, 2008).

No evidence of teratogenicity was seen in studies in the rat and rabbit, and a developmental NOAEL of 120 - 250 mg/kg bw/day in rabbits and 250 - 450 mg/kg bw/day in rats have been proposed (EFSA, 2008).

4.2 Metazachlor sulfonic acid

Searches were made for publicly available information on the toxicity of metazachlor sulfonic acid, including use of the online programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1 Predicted toxicity data for metazachlor sulfonic acid using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$ (range)				
Metazachlor (experimental data)	2000	34.5	NOAEL: 8.5 mg/kg/day Main target organ: Liver	No evidence	Thyroid parafollicular adenomas and carcinomas in rats	Reduced litter size, pup weight and survival (rats).
Metazachlor sulfonic acid (TOPKAT)	UE	10,000 (10,000-10,000)	MTD (feed/drink) 2.3 mg/kg MTD (oral gavage) 6.3 mg/kg	Negative	Negative	Predicted developmental toxicant
Metazachlor sulfonic acid (DEREK)	n/a	n/a	*Plausible* for skin sensitisation in humans	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software; U – unreliable estimate

DEREK identified the presence of only one structural alert and the following conclusion was drawn:

It is considered *plausible* that metazachlor sulfonic acid will exhibit skin sensitisation in humans. This endpoint is predicted because metazachlor sulfonic acid is a formaldehyde donor.

The analysis by TOPKAT suggested that metazachlor sulfonic acid was:

non-carcinogenic;
non-mutagenic; but
a developmental toxicant.

5 Guidelines and Standards

5.1 Metazachlor

The EC risk classification is: Xi – Irritant: R34; N – Dangerous for the environment: R50, R53. The EC safety classification is: S24, S37, S60, S61. Metazachlor is classified by WHO as 'unlikely to present acute hazard in normal use' while no consensus on classification was reached by the US EPA (Lewis *et al.,* 2007).

An ADI of 0.08 mg/kg bw/d has been proposed for metazachlor, with a safety factor (SF) of 100, based on a two year rat study in which Wistar rats received metazachlor via the diet at up to 8000 ppm (EFSA, 2008; Lewis *et al*., 2007). Although evidence of carcinogencity was noted in this study, a SF of 100 was adopted because this end point was not considered to be relevant to humans (Hunter, 1983).

5.2 Metazachlor sulfonic acid

Due to the lack of availabile human or experimental data, it has not been possible to establish a robust NOEL or NOAEL for metazachlor sulfonic acid. In addition, no ADI has been proposed for this metabolite by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The predicted toxicity profile for metazachlor sulfonic acid suggests that it may be slightly less active than the parent. The highest oral MTD predicted by TOPKAT for metazachlor sulfonic acid is 6.3 mg/kg bw/day (for oral gavage administration); this is similar to the NOAEL of 8.5 mg/kg bw/day established for the parent compound.

Applying a SF of 100 to the metabolites predicted MTD would give a nominal value of 0.063 mg/kg bw/day; this is similar to the established ADI of 0.08 mg/kg bw/day for the parent metazachlor. Therefore, – on a highly precautionary basis – it is proposed to adopt a PSDV of 0.08 mg/kg bw/day for metazachlor sulfonic acid (i.e. the same value as for the parent).

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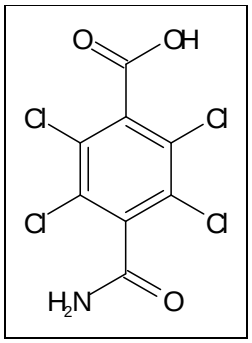
Appendix 12.3 3-Carbamyl-1,2,4,5-tetrachlorobenzoic acid

1 Introduction

3-carbamyl-1,2,4,5-tetrachlorobenzoic acid is a metabolite of the fungicide chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile; CAS No. 1897-45-6).

The structure of 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid is presented in Figure 1.

Figure 1: Structure of 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Chlorothalonil

Chlorothalonil is a broad-spectrum non-systemic pesticide that acts primarily as fungicide and mildewicide, but additionally has some activity as a bactericide, microbiocide, algaecide, insecticide and acaricide. Chlorothalonil was first registered in the US in 1966 for application to turf, but is now registered for a wide range of sites including, field, vegetables and orchard crops; turf; and as a mildewicide in paint and other surface treatments (US EPA, 1999).

Chlorothalonil is currently sold worldwide under several commercial names including, Bravo, Daconil 2787, Echo, Exotherm Termil, Forturf, Mold-Ex, Nopcocide N-96, Ole, Pillarich, Repulse and Tuffcide; in general it is soluble concentrate that is mixed with water and applied as a spray. Application rate varies among crops; most crops have a maximum rate of application of between 0.28 – 0.41 kg chlorothalonil per hectare, with two crops having higher use (0.83 – 0.92 kg chlorothalonil per hectare; ENVIRO*facts*, 2003).

2.2 Environmental fate

2.2.1 Parent

During application, any chlorothalonil released into the air is likely to exist in both vapour and particulate phases. Chlorothalonil within the vapour phase will be photochemically degraded with an estimated half-life of 7 years (HSDB, 2006); although photolysis may also occur in the particulate phase, the rate of this is not known.

Particulate-phase chlorothalonil is removed from the atmosphere by wet and dry deposition. Chlorothalonil has low water solubility (0.81 mg/L at 25^{o}C, pH (neutral); EC, 2006) and, in assessing the sorption to soil, K_{oc} values of between 900 and 7000 L kg^{-1} have been described (HSDB, 2006), suggesting slight, or no mobility.

Chlorothalonil is moderately susceptible to degradation in soil under aerobic conditions (US EPA, 1999), with degradation occurring mainly through dechlorination and some substitution reactions (Sato et al., 1987). The degradation half-life

measured in four different soils has been reported as between 10 and 40 days (HSDB, 2006), with an average half-life estimated as 22 days (Lewis *et al*., 2007). Chlorothalonil is not considered to be persistent.

2.2.2 Metabolite: 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid

No information relating to either the physicochemical properties or environmental fate of 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid was identified.

2.3 Potential routes of human exposure

During production Chlorothalonil may be released to the environment through various waste streams. Direct release into the environment will also occur during its use as a pesticide. Exposure to chlorothalonil may occur through ingestion of contaminated food and water or through inhalation or dermal contact during occupational handling or by-stander exposure (HSDB, 2006).

Although no data on routes of exposure of humans to the metabolite 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid was identified, it is plausible that exposure may occur as a result of its ingestion in food and water contaminated from the breakdown of chlorothalonil (oral route) or when formed as a human metabolite from ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of chlorothalonil in humans.

Toxicokinetic studies for Chlorothalonil have been carried in Sprague-Dawley rats orally dosed with ^{14}[C]-chlorothalonil at 5, 50 and 200 mg/kg). In males approximately 89% of the dose was excreted, and in females approximately 96%. The major route of excretion was the faeces (83-87%) with excretion apparently complete within 48 hr in low-dose females and low and mid-dose males, and by 72 hr in mid/high dose females and high dose males. Some urinary excretionj also occurred which showed saturation at higher doses. At low doses, urinary excretion was 92-93% complete within 24hr, and for mid doses within 48 hr, however, at high doses 95% excretion was achieved by 72 hr (IPCS, 1996).

A similar pattern was noted in rats given a single low oral dose (1.5mg/kg) of chlorothalonil. Around 30 – 32% of administered dose was absorbed from the GI tract, with approximately 20 – 22% of the absorbed dose being excreted in bile and a further 10% excreted in urine (Krieger, 2001).

In monkeys treated dermally with 5 mg/kg bw of ^{14}[C]-chlorothalonil under a non-occlusive patch for 48 hr, around 90% of the dose was recovered from the surface, i.e. unabsorbed; only 2.26% was absorbed through the skin. Urine contained 1% of the absorbed dose but this did not include any detectable methylated mono-, di- and trithiols (IPCS, 1996). In male Sprague-Dawley rats given 200 mg/kg ^{14}[C]-chlorothalonil by oral gavage, urine collected at 17, 24 and 48 hr following administration contained the metabolites trimethylthiomonochloro-isophthalonitrile and dimethylthiodichloro-isophthalonitrile, excreted as free thiols or methylated derivatives, and accounted for 2.4% of the administered dose (IPCS, 1996).

In a repeat oral dose study, male rats were given ^{14}[C]-chlorothalonil at 1.5, 5, 50 or 160 mg/kg/day, and culled 2, 9, 24, 96 and 168 hr following administration of the last dose. Chlorothalonil was found to be widely distributed, with highest concentrations occurring in kidneys, followed by liver and blood. Peak concentrations were seen 2 hr

after the last dose, at which time 0.28% and 0.20% of the radioactivity was found in the kidney for the 1.5 and 5 mg/kg dose levels respectively (IPCS, 1996).

In a further study, rats were given repeated doses of ^{14}[C]-chlorothalonil at up to 160 mg/kg/day. Methylated or partly methylated dithiol and trithiol derivatives were identified in urine at all doses (IPCS, 1996).

No information on the toxicokinetics of 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid was identified.

4 Toxicity Profile

4.1 Chlorothalonil

4.1.1 Acute Toxicity

No information is available on the acute toxicity of chlorothalonil to humans.

Chlorothalonil has low acute toxicity in rats, with LD_{50}'s of 5000 mg/kg, 2000 mg/kg and 0.1 mg/m^3 when given by the oral, dermal or inhalation routes, respectively (EC, 2006).

Chlorothalonil is a contact irritant to the skin, eye and respiratory tract (US EPA, 1999) and there is evidence to suggest that it is a skin sensitisation (EC, 2006).

4.1.2 Repeat dose toxicity

Repeated exposure of humans to chlorothalonil has been associated with development of contact dermatitis and other skin conditions. For example, employees in a chlorothalonil manufacturing plant were reported to have an increased incidence (60%) of skin abnormalities when compared with non-exposed workers (18.5%; WHO, 1996).

In animal studies, the principal target organs of repeated exposure for Chlorothalonil are the kidney and fore-stomach. In a 2 year feeding study, Fischer-344 rats were administered chlorothalonil via the diet at up to 175mg/kg bw/day. Histopathological examination of the kidneys showed nephropathy, tubular hyperplasia (considered a preneoplastic change) and chronic progressive nephropathy in rats of treated groups. Increased incidence and severity of hyperplasia and hyperkeratosis of the squamous mucosa of the oesophagus and forestomach was also noted in treated groups; the stomach and oesophageal changes were attributed to an irritant effect of chlorothalonil. A NOEL of 1.8 mg/kg bw/ day was established for non-neoplastic effects (IPCS, 1996).

Similar findings were noted in Beagle dogs administered chlorothalonil at dietary levels up to 750 mg/kg bw/day for 2 years, with treatment-related histopathological changes being seen in the liver, thyroid, kidney and stomach at mid and high doses (IPCS, 1996).

4.1.3 Carcinogenicity and mutagenicity

There is inadequate evidence for the carcinogenicity of chlorothalonil in humans. However, IARC has noted that there is *sufficient evidence of carcinogenicity in experimental animals*, and concluded that chlorothalonil is *possibly carcinogenic to humans* (Group 2B; IARC, 1999).

In the 2 year dietary study, described above, in which Fischer-344 rats were administered chlorothalonil at up to 175 mg/kg bw/day, the incidence of renal tubular adenoma and carcinoma was increased in treated male and female groups. A

treatment-related increase in incidence and severity of renal tubular epithelial hyperplasia was also noted and was considered a pre-neoplastic change. Hyperplasia and hyperkeratosis of the squamous mucosa of the oesophagus and forestomach were also apparent (IPCS, 1996).

The carcinogenic potential of Chlorothalonil administered in the diet at doses up to 165 mg/kg bw/day was assessed in a further rat study; a NOEL of 3.8 mg/kg d was established.

Evidence on the genotoxic potential of chlorothalonil is conflicting. Although some *in vitro* models without activation have shown positive results, the available *in vivo* studies were inconclusive (EC, 2006; HSDB, 2006).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of chlorothalonil in humans was identified.

In a two-generation reproduction study in Charles River CD rats, chlorothalonil was administered via the diet at up to 3000 mg/kg diet. No treatment-related effects were noted in F_0 or F_1 parent animals and reproductive parameters were unaffected. However pups displayed lowered bodyweights. A NOEL for this effect of 75 mg/kg bw/day was proposed (IPCS, 1996).

Similar results have been described in rabbits when given chlorothalonil by oral gavage at up to 20 mg/kg bw/day on days 7-19 of gestation. A lowered maternal body weight in conjunction with decreased food consumption was noted at the highest dose; pregnancy rate was $\geq$ 95% in all groups and no evidence of fetotoxicity or teratogenicity was found (IPCS, 1996).

4.2 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid

4.2.1 Acute toxicity

No information on the acute toxic effects of 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid in humans was identified.

4.2.2 Repeat dose toxicity

No information on the repeat dose toxicity of 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid in humans was identified.

Searches were made for publicly available information on the toxicity of 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid, including use of the online programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK identified the presence of structural alerts and the following conclusions were drawn:

It is considered equivocal that 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid will exhibit alpha-2-μ-globulin nephropathy in mammals. This endpoint is predicted because 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid is a polyhalogenated benzene compound.

It is considered equivocal that 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid will exhibit carcinogenicity in humans. This endpoint is predicted because 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid is a polyhalogenated aromatic compound

The analysis by TOPKAT suggested that 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid was:

non-carcinogenic;
non-mutagenic; but
a developmental toxicant.

Table 1: Predicted toxicity data for 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity/ Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Chlorothalonil (experimental data)	5000	0.1	NOAEL: 1.8 mg/kg/day Main target organs: kidney and fore-stomach	Genotoxic potential not established	Fore-stomach tumours in rats and mice, kidney tumours in mice	Reduced pup weight (rats).
3-carbamyl-1,2,4,5-tetrachloroben-zoic acid (TOPKAT)	6000 (1200-10,000)	7200 (918.1-10,000)	MTD (feed/drink) 193 mg/kg MTD (oral gavage) 193 mg/kg	Negative	Negative	Predicted developmental toxicant
3-carbamyl-1,2,4,5-tetrachloroben-zoic acid (DEREK)	n/a	n/a	*Equivocal* for alpha-2-µ-globulin nephropathy in mammals	No alert	*Equivocal* for carcinogenicity in humans	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software

5 Guidelines and Standards

5.1 Chlorothalonil

The EC risk classification is: Carcinogen category 3: R40; T+ - very toxic: R26; Xi – Irritant: R37, R41, R43; N – Dangerous for the environment: R50, R53.Chlorothalonil is classified by WHO as 'unlikely to present acute hazard in normal use' and by the US EPA as 'moderately toxic' (Lewis *et al.,* 2007).

An ADI of 0.018 mg/kg bw/d has been established for chlorothalonil, using a safety factor (SF) of 100, based on a two year rat study in which Fischer 344 rats received chlorothalonil via the diet at levels up to 175 mg/kg bw/day (IPCS, 1996; Lewis *et al*., 2007).

5.2 3-Carbamyl-1,2,4,5-tetrachlorobenzoic acid

Due to the lack of available human or experimental data, it has not been possible to establish a robust NOEL or NOAEL for 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid. In addition, no ADI has been proposed for this metabolite by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The predicted toxicity profile for 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid suggests that it may be slightly less active than the parent. The highest oral MTD predicted by TOPKAT for 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid is 193 mg/kg bw (irrespective of form of oral administration). This is significantly higher than the NOEL of 1.8 mg/kg bw/day established for the parent compound.

Applying a SF of 100 to the metabolites predicted MTD would give a nominal value of 1.93 mg/kg bw. This is significantly above the established ADI of 0.018 mg/kg/bw for the parent chlorothalonil. Therefore, – on a highly precautionary basis – it is proposed to adopt a PSDV of 0.018 mg/kg bw/d for 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid (i.e. the same value as for the parent); this will provide an overall SF of >10,000.

6 References

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Appendix 12.4 SDS-46851 (3-Carbamyl-2,4,5-trichlorobenzoic acid)

1 Introduction

3-carbamyl-2,4,5-trichlorobenzoic acid (SDS-46851) is a major metabolite of the fungicide chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile; CAS No. 1897-45-6). It is formed within soil and through metabolism of absorbed chlorothalonil in humans.

2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of chlorothalonil

Chlorothalonil is a broad-spectrum non-systemic pesticide that is used primarily as a fungicide and mildewicide but has some additional activity as a bactericide, microbiocide, algaecide, insecticide and acaricide. Chlorothalonil was first registered in the US in 1966 for application to turf but is now registered for a wide range of applications including on fields, vegetables and orchard crops and as a mildewicide in paint and other surface treatments (US EPA, 1999).

Chlorothalonil is currently sold worldwide under several commercial names including Bravo, Daconil 2787, Echo, Exotherm Termil, Forturf, Mold-Ex, Nopcocide N-96, Ole, Pillarich, Repulse and Tuffcide. In general it is sold as a soluble concentrate that is mixed with water and applied as a spray. Application rates vary depending on use. For most crops the maximum rate of application is between 0.28 – 0.41 kg chlorothalonil per hectare although two crops require a higher rate of 0.83-0.92 kg chlorothalonil per hectare (ENVIRO*facts*, 2003).

2.2 Environmental fate

2.2.1 Parent

Any chlorothalonil released into the air during application is likely to enter both vapour and particulate phases. Chlorothalonil within the vapour phase will be slowly photochemically degraded with an estimated half-life of 7 years (HSDB, 2006).

Although photolysis might also occur in the particulate phase, no data on the rate at which this might occur is available. Particulate-phase chlorothalonil is mainly removed from the atmosphere by wet and dry deposition.

Chlorothalonil has low water solubility (0.81 mg/L at 25^{o}C, pH (neutral); EC, 2006). K_{oc} values of between 900 and 7000 L kg^{-1} have been described (HSDB, 2006) suggesting slight or no mobility to soils.

Chlorothalonil is moderately susceptible to degradation in soil under aerobic conditions (US EPA, 1999). Degradation occurs mainly through dechlorination and some substitution reactions (Sato et al., 1987). The degradation half-life in four soils has been reported to be between 10 and 40 days (HSDB, 2006); the average half-life was 22 days (Lewis *et al*., 2007). Chlorothalonil is not considered to be persistent.

2.2.2 Metabolite: SDS-46851

SDS-4685 is one of the major metabolites formed from chlorothalonil, with an estimated maximum formation fraction of 0.132 (13%; Lewis *et al*., 2007).

A K_{oc} value of 77 L kg^{-1} has been reported for SDS-46851 (Lewis *et al*., 2007) suggesting it has moderate mobility in the environment.

The degradation half-life of SDS-46851 in soil under aerobic conditions is estimated to be 103 days and the metabolite is considered to be persistent (Lewis *et al.,* 2007).

No further information on the physicochemical properties or environmental fate of SDS-46851 was identified.

2.3 Potential routes of human exposure

During production Chlorothalonil may be released to the environment through various waste streams. Direct release into the environment will also occur during its use as a pesticide. Exposure to chlorothalonil may occur through ingestion of contaminated food and water or through inhalation or dermal contact during occupational handling or by-stander exposure (HSDB, 2006).

Although no data on routes of exposure of humans to the metabolite SDS-4685 was identified, it is plausible that exposure may occur as a result of its ingestion in food and water contaminated from the breakdown of chlorothalonil (oral route) or when formed as a human metabolite from ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of chlorothalonil in humans.

Toxicokinetic studies have been carried in Sprague-Dawley rats orally dosed with ^{14}[C]-chlorothalonil at 5, 50 or 200 mg/kg. In males, approximately 89% of the radioactive dose was excreted and in females approximately 96%. The major route of excretion was the faeces (83 - 87%) with excretion apparently complete within 48 hr in low dose females and low and mid dose males, and by 72 hr in mid/high dose females and high dose males. Some urinary excretion also occurred which showed saturation at higher doses. At low doses, urinary excretion was 92 - 93% complete within 24 hr, and for mid doses within 48 hr, however, at high doses only 95% excretion was achieved by 72 hr (IPCS, 1996).

A similar pattern was noted in rats given a single low oral dose (1.5mg/kg) of chlorothalonil. Around 30 - 32% of administered dose was absorbed from the GI tract, with approximately 20 - 22% of the absorbed dose being excreted in bile, and a further 10% excreted in urine (Krieger, 2001).

In monkeys treated dermally with 5 mg/kg bw of ^{14}[C]-chlorothalonil under a non-occlusive patch for 48 hr, around 90% of the dose was recovered from the treated surface, i.e. unabsorbed; only 2.26% was absorbed through the skin. Urine contained 1% of the absorbed dose but this did not include any detectable methylated mono-, di- and trithiols (IPCS, 1996). In male Sprague-Dawley rats given 200 mg/kg ^{14}[C]-chlorothalonil by oral gavage, urine collected at 17, 24 and 48 hr following administration contained the metabolites trimethylthiomonochloro-isophthalonitrile and dimethylthiodichloro-isophthalonitrile, excreted as free thiols or methylated derivatives, and accounted for 2.4% of the administered dose (IPCS, 1996).

In a repeat oral dose study male rats were given ^{14}[C]-chlorothalonil at 1.5, 5, 50 or 160 mg/kg/day, and culled 2, 9, 24, 96 and 168 hr following administration of the last dose. Chlorothalonil was found to be widely distributed, with highest concentrations in the kidneys followed by liver and blood. Peak concentrations were seen 2 hr after the last dose, at which time 0.28% and 0.20% of the radioactivity was found in the kidney for the 1.5 and 5 mg/kg dose levels respectively (IPCS, 1996).

In a further study, rats were given repeated doses of ^{14}[C]-chlorothalonil at up to 160 mg/kg/day. Methylated or partly methylated dithiol and trithiol derivatives were identified in urine at all doses (IPCS, 1996).

In a study in rats, oral absorption of SDS-46851 was between 22 - 26%, based on urinary excretion (EC, 2006; from non-published, data-protected company reports).

In a further study on Sprague-Dawley rats administered a single oral dose of ^{14}[C]-chlorothalonil at up to 200 mg/kg, levels of SDS-46851 in urine corresponded to 3.5% and 4.5% of the applied dose at 200 and 5 mg/kg, respectively. In faeces, levels of this metabolite were 1.4 and 2.5% of applied dose (PSD, 1994).

No further information on the toxicokinetics of SDS-46851 was identified.

4 Toxicity Profile

4.1 Chlorothalonil

4.1.1 Acute Toxicity

No information is available on the acute toxicity of chlorothalonil to humans.

Chlorothalonil has low acute toxicity in rats; LD_{50}'s are 5000 mg/kg, 2000 mg/kg and 0.1 mg/m^3 when given by oral, dermal or inhalation routes respectively (EC, 2006).

Chlorothalonil is a contact irritant to the skin, eye and respiratory tract (US EPA, 1999) and there is evidence to suggest that it is a skin sensitiser (EC, 2006).

4.1.2 Repeat dose toxicity

Repeated exposure of humans to chlorothalonil has been associated with development of contact dermatitis and other skin conditions. For example, employees in a chlorothalonil manufacturing plant were reported to have an increased incidence (60%) of skin abnormalities compared with non-exposed workers (18.5%; IPCS, 1996).

In animal studies, the principal target organs of repeated exposure are considered to be the kidney and forestomach.

In a 2 year feeding study, Fischer-344 rats were administered chlorothalonil via the diet at up to 175mg/kg bw/day. Histopathological examination of the kidneys showed nephropathy, tubular hyperplasia (considered a preneoplastic change) and chronic progressive nephropathy in rats of treated groups. Increased incidence and severity of hyperplasia and hyperkeratosis of the squamous mucosa of the oesophagus and forestomach were also noted in treated groups; the stomach and oesophageal changes were attributed to an irritant effect of chlorothalonil. A NOEL of 1.8 mg/kg bw/day was established for non-neoplastic effects (IPCS, 1996).

Similar findings were noted in Beagle dogs administered chlorothalonil at dietary levels up to 750 mg/kg bw/day for 2 years, with treatment-related histopathological changes being seen in the liver, thyroid, kidney and stomach at mid and high doses (IPCS, 1996).

4.1.3 Carcinogenicity and mutagenicity

There is inadequate evidence of carcinogenicity of chlorothalonil in humans. However, IARC has noted that there is *sufficient evidence of carcinogenicity in experimental animals*, and concluded that chlorothalonil is *possibly carcinogenic to humans* (Group 2B; IARC, 1999).

In the two year dietary study in which Fischer-344 rats were administered chlorothalonil at up to 175 mg/kg bw/day described above, the incidence of renal tubular adenoma and carcinoma was increased in treated male and female groups. A

treatment related increase in incidence and severity of renal tubular epithelial hyperplasia was also noted and was considered a pre-neoplastic change. Hyperplasia and hyperkeratosis of the squamous mucosa of the oesophagus and forestomach were also apparent (IPCS, 1996).
The carcinogenic potential of chlorothalonil administered in the diet at doses up to 165 mg/kg bw/day was assessed in a further rat study; a NOEL of 3.8 mg/kg bw/day was established (IPCS, 1996).

Evidence on the genotoxic potential of chlorothalonil is conflicting. Although some *in vitro* models without activation have shown positive results, the available *in vivo* studies were inconclusive (EC, 2006; HSDB, 2006).

4.1.4 Reproductive and development toxicity
No information on the reproductive and developmental toxicity of chlorothalonil in humans was identified.

In a two-generation reproduction study in Charles River CD rats, chlorothalonil was administered via the diet at up to 3000 mg/kg diet. No treatment-related effects were noted in F_0 or F_1 parent animals and reproductive parameters were unaffected. However pups displayed lower bodyweights. A NOEL for this effect of 75 mg/kg bw/day was proposed (IPCS, 1996).

Similar results have been described in rabbits when given chlorothalonil by oral gavage at up to 20 mg/kg/day on days 7 - 19 of gestation. A lowered maternal body weight in conjunction with decreased food consumption was noted at the highest dose; pregnancy rate was $\geq$ 95% in all groups and no evidence of fetotoxicity or teratogenicity was found (IPCS, 1996).

4.2 SDS-46851
4.2.1 Acute Toxicity
No information on the acute toxic effects of SDS-46851 in humans was identified.

In rats, SDS-46851 has low acute toxicity with an oral LD_{50} of > 5000 mg/kg (EC, 2006).

4.2.2 Repeat dose toxicity
No information on the repeat dose toxicity of SDS-46851 was identified.

Experimentally, a NOAEL of 200 mg/kg bw/day has been proposed for SDS-46851 in rats (EC, 2006; from non-published, data-protected company report).

4.2.3 Carcinogenicity and mutagenicty
SDS-46851 has been reported to show neither carcinogenic or genotoxic potential in experimental studies (EC, 2006; from non-published, data-protected company reports).

4.2.4 Reproductive and developmental toxicity
At parental toxic doses, no effect on reproductive endpoints were noted in a study in rats; a reproductive NOAEL of > 911 mg/kg bw/day was proposed (EC, 2006; from non-published, data-protected company report).

In developmental studies, administration of SDS-46851 at maternal toxic doses resulted in a decrease in numbers of live fetuses and a decrease in fetal weight; a

developmental NOAEL of 500 mg/kg bw/day was proposed (EC, 2006; from non-published, data-protected company report).

Further searches were made for publicly available information on the toxicity of SDS-46851, including use of the online programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.
Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK identified the presence of structural alerts and the following conclusions were drawn:

It is considered equivocal that SDS-46851 will exhibit alpha-2-mu-Globulin nephropathy in mammals. This endpoint is predicted because SDS-46851 is a polyhalogenated benzene compound.
It is considered equivocal that SDS-46851 will exhibit carcinogenicity in humans. This endpoint is predicted because SDS-46851 is a polyhalogenated aromatic compound.

The analysis by TOPKAT suggested that SDS-46851 was:

non-carcinogenic; and
non-mutagenic.

Assessment of reproductive and developmental toxicity using TOPKAT was not possible for the metabolite.

Table 1: Predicted toxicity data for SDS-46851 using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H(range)				
Chlorothalonil (experimental data)	5000	0.1	NOAEL: 1.5 mg/kg/day Main target organs: kidney and fore-stomach	Genotoxic potential not established	Fore-stomach tumours in rats and mice, kidney tumours in mice	Reduced pup weight (rats).
SDS-46851 (experimental data)	> 5000	n/d	200 mg/kg bw/day	Negative	Negative	Decreased live fetuses: Reproductive NOAEL of 911 mg/kg bw/day Decreased fetal weight: Developmental NOAEL of 500 mg/kg bw/day.
SDS-46851 (TOPKAT)	UE	5300 (681.5-10,000)	MTD (feed/drink) 1800 mg/kg MTD (oral gavage) 1800 mg/kg	Negative	Negative	Indeterminate
SDS-46851 (DEREK)	n/a	n/a	*Equivocal* for alpha-2-µ-globulin nephropathy in mammals	No alert	*Equivocal*	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software; UE – unreliable estimate

5 Guidelines and Standards

5.1 Chlorothalonil

The EC risk classification is: Carcinogen category 3: R40; T+ - very toxic: R26; Xi – Irritant: R37, R41, R43; N – Dangerous for the environment: R50, R53. Chlorothalonil is classified by WHO as 'unlikely to present acute hazard in normal use' and by the US EPA as 'moderately toxic' (*Lewis et al.,* 2007).

An ADI of 0.018 mg/kg bw/d has been established for chlorothalonil using a safety factor (SF) of 100, based on a two year rat study in which Fischer 344 rats received chlorothalonil via the diet at up to 175 mg/kg/d (IPCS, 1996; Lewis *et al*., 2007).

5.2 SDS-46851

SDS-46851 is not listed under EC, WHO or US EPA classifications.

An ADI of 0.2 mg/kg bw/d has been proposed for SDS-46851 using a SF of 100, based on a study in rats (EC, 2006 from non-published, data-protected company report). Although the overall reliability of the study could not be assessed here, in view of the low concern also identified by the predictive software systems, this value will be adopted in the risk assessment.

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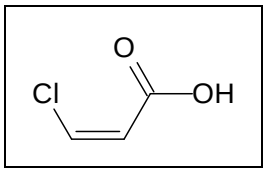
Appendix 12.5 cis-3-chloroprop-2-enoic acid

1 Introduction

cis-3-chloroprop-2-enoic acid is a major metabolite of the nematicide 1,3 dichloropropene (CAS No. 542-75-6), that is formed within the plant, surrounding soil and groundwater. This metabolite is also formed through metabolism of 1,3-dichloropropene in humans.

The structure of cis-3-chloroprop-2-enoic acid is presented in Figure 1.

Figure 1: Structure of cis-3-chloroprop-2-enoic acid



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of 1,3-dichloropropene

1,3-dichloropropene is a general biocide comprised of cis- and trans-isomers. It is used primarily as a nematicide but also against certain plant diseases and in the control of insects and weeds on potatoes, tomatoes, tobacco, pineapple, and other vegetable and orchard crops. Typical usage rates are up to 280 kg/ha (Lewis *et al,* 2007).

1,3-dichloropropene is currently registered for use in the EU, Asia, South Africa, Pacific Region and North America under several commercial names including: Telone(R)Dowfume N; Telone C-17(R)Vidden D; Telone II(R)Terr-O-Cide 15-D; Telone II(R) bTerr-O-Cide 30-D; DD(R)Terr-O-Gas 57/43T; DD-92(R)Vorlex; M-3993 D-D 95; Dedisol C Nematox II; and Telone 2000 (IPCS, 1997). In general it is formulated as a colourless liquid and applied to the soil via drip irrigation systems or soil injection prior to planting (Lewis *et al*., 2007).

On 20th September 2007 the European Commission published a decision to withdraw existing authorisations for plant protection products containing 1,3-dichloropropene by March 2008, with no new authorisations subsequently being granted. A period of grace expiring no later than March 2009 was allowed (EC, 2007). A number of areas of concern were identified, particularly with regards to the release of unknown polychlorinated impurities into the environment, for which information on persistency, toxicology, uptake from crops, accumulation and metabolic fate was not available.

2.2 Environmental fate

2.2.1 Parent

1,3-dichloropropene is highly soluble in water (2485 mg/L at 20^{o}C, Lewis *et al*., 2007). In assessing the sorption to soil, a K_{oc} value of 34 L kg^{-1} has been described (Lewis *et al*., 2007), suggesting that the pesticide will be mobile in the environment.

The volatility of 1,3-dichloropropene is high and approximately 5 to 75% will volatise within 36 hr after soil injection. Degradation of 1,3-dichloropropene in the atmosphere occurs as a result of reactions with hydroxyl radicals, with a half-life of between 1 and 50 hr. It is not considered to be persistent in the atmosphere (US EPA, 2000).

1,3-dichloropropene is relatively rapidly degraded in soil with a degradation half-life of between 4.6 and 13.5 days under laboratory conditions (at 20ºC) and between 1 and 7 days under field conditions (IPCS, 1997).

2.2.2 Metabolite: cis-3-chloroprop-2-enoic acid

cis-3-chloroprop-2-enoic acid is one of the major metabolites formed following degradation of 1,3-dichloropropene, with an estimated formation fraction of 0.373 (37%; Lewis *et al*., 2007).

A K_{oc} value of 9.4 L kg^{-1} has been reported for cis-3-chloroprop-2-enoic acid (Lewis *et al*., 2007) suggesting that the metabolite is very mobile in the environment.

The degradation half-life of cis-3-chloroprop-2-enoic acid in soil under laboratory conditions is 12.4 days; the metabolite is not considered to be persistent in soil (Lewis *et al.,* 2007).

2.3 Potential routes of human exposure

Potential routes of human exposure to 1,3-dichloropropene include inhalation, ingestion of contaminated drinking water and through dermal contact. The pesticide is usually not present in food as it has a short environmental persistence.

High levels of exposure to 1,3-dichloropropene are most likely to occur in occupational settings, either during production or use of the pesticide. Inhalation or dermal contact are the most probable routes of exposure in the workplace (ATSDR, 2008).

Although no data on routes of exposure of humans to the metabolite cis-3-chloroprop-2-enoic acid was identified, it is plausible that exposure may occur as a result of its ingestion in food or water contaminated from the breakdown of dichloropropene, or when formed as a human metabolite from ingestion of the parent compound.

3 Toxicokinetics

There are limited data with which to assess absorption following exposure of humans to 1,3-dichloropropene. Although no studies were identified relating to the oral absorption of 1,3-dichloropropene in humans, in one study, six male volunteers exposed to 1 ppm Telone II via inhalation for six hours showed between 72 and 80% absorption of the cis-isomer of 1,3-dichloropropene (ATSDR, 2008).

Dermal absorption was assessed through exposure of forearm skin in volunteers to cis-1,3-dichloropropene vapor at a concentration of 86 mg/m^3 (19 ppm) for 45 minutes. Recovery in urine (assessed by presence of the metabolite cis-1,3-dichloropropene-mercapturic acid) over a 20 hr period indicated between 2 - 5% absorption (Kezic *et al*., 1996).

Animal studies of the absorption of 1,3-dichloropropene following inhalation support findings in humans. In rats exposed to vapour concentrations of up to 900 ppm, absorption ranged from 82% to 62% (low to high exposures; Stott & Kastl, 1986). 1,3-dichloropropene was also easily absorbed following gavage dosing of ^{14}[C]-cis/ trans-1,3-dichloropropene in rats, with recovery of radioactivity in urine over a 24 hr period amounting to 82 - 84% (Climie et al., 1979). No studies were found relating to the absorption of 1,3-dichloropropene following dermal exposure in animals.

The distribution of 1,3-dichloropropene in humans following its inhalation at 1 ppm for 6 hr was assessed in six volunteers. Blood concentrations reached an apparent plateau within one hr of exposure, with levels of the cis-isomer ranging from 0.3 – 2 ppb (Waechter et al., 1992).

Experimentally, administration of ^{14}[C]-cis/trans-1,3-dichloropropene to rats by oral gavage resulted in a uniform concentration of radioactivity in most organs and tissues after 48 hr (Waechter & Kastl, 1988). The highest concentration of radioactivity was in the nonglandular stomach and the urinary bladder. No studies relating to the distribution of any isomer of dichloropropene after dermal exposure in humans or animals were found.

1,3-dichloropropene is extensively metabolised although the rate of metabolism differs for the two isomers (Stott & Kastl, 1986). The metabolic pathway is common for all routes of exposure, and involves the rapid conjugation of 1,3-dichloropropene with glutathione in the liver, forming a mercapturic acid metabolite that is subsequently excreted via the kidney. Minor alternative pathways involve hydrolysis and dechlorination of the parent to form 1-chloroallyl alcohol or reaction with cytochrome P450 to form the mutagenic cis- and trans-epoxides that convert to 3-chloro2- hydroxy-propanal which is itself mutagenic (Schneider *et al*., 1998). ATSDR (2008) states that metabolic pathways are similar in rats and humans.

As noted above, the main excretory route is via urine. No studies relating to excretion of 1,3-dichloropropene following oral exposure in humans were identified. However, conjugates of cis-1,3-dichloropropene have been detected in the urine of four men occupationally exposed to Telone II®a at levels that correlate with exposure (Osterloh *et al*., 1984). The mercapturic acid metabolite of cis-1,3-dichloropropene was also detected in the urine of volunteers following dermal exposure (forearm skin) to a vapour concentration of 86 mg/m^3 (19 ppm) for 45 minutes (Kezic *et al*., 1996). In male volunteers exposed by inhalation to 1 ppm 1,3-dichloropropene for 6 hr, levels of parent compound plateaued in exhaled air by one hr and were no longer detectable one hr following the end of exposure. Urinary levels of N-acetyl-cysteine showed a biphasic pattern with half-lives of 4.2 - 3.2 hr during the initial phase and 12.3 - 17.1 hr during the second phase (Waechter *et al.,* 1992). The half-life for urinary excretion of the mercapturic acid metabolite following dermal exposure is reported to be approximately 6 hr (Kezic *et al.,* 1996).

Experimentally, a bi-phasic excretion has also been shown in male Fischer rats following inhalation exposure to 1,3-dichloropropene at 30, 90, 300 or 900 ppm for 3 hr. At doses up to 300 ppm, the half-life for the initial phase was 3 - 6 minutes, while that for the second phase was 33 - 43 minutes. At the highest dose, the initial half-life extended to 14 - 27 minutes (Stott & Kastl, 1986). The pattern of clearance following oral dosing of rats with 1,3-dichloropropene was reported to be similar (Stott *et al*., 1998).

No studies relating to excretion of 1,3-dichloropropene after dermal exposure in animals were identified.

No information on the toxicokinetics of cis-3-chloroprop-2-enoic acid was identified.

4 Toxicity Profile
4.1 1,3-dichloropropene
4.1.1 Acute Toxicity

Acute exposure of humans to 1, 3-dichloropropene through inhalation has been anecdotally associated with a range of effects, most importantly the development of non-Hodgkin's (histiocytic) lymphoma and acute myelomonocytic anaemia (Markovitz &Crosby 1984), as well as a range of non-specific symptoms such as headache, nausea, vomiting, fatigue, impotence and malaise (Flessel *et al*., 1978; Markovitz & Crosby, 1984). The most common findings following inhalation exposure are chest discomfort, breathing difficulties, coughing and mucous membrane irritation. Concentrations of 1, 3-dichlorpropene in excess of 1000 ppm may also cause lacrimation (ACGIH, 1989).

Ingestion of 1,3-dichloropropene has been reported to cause death with gastrointestinal, respiratory and cardiac effects preceding a multi-organ failure; at the time of death levels of 1,3-dichloropropene in blood and urine were 1.13 µmol/L and 0.20 µmol/L respectively (Hernandez *et al*., 1994). Delayed-type hypersensitivity reactions have also been reported in humans following dermal exposure (ATSDR, 2008).

Experimentally, 1,3-dichloropropene is moderately to highly toxic in rats; LD_{50}'s are 130 mg/kg, 333 mg/kg and 2.7 mg/m^3 by the oral, dermal and inhalation routes respectively (Lewis *et al*., 2007).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of 1, 3-dichloropropene in humans was identified.

A dietary study in which F344 rats were administered Telone II at up to 25 mg/kg/ bw/day for two years (Stott *et al*.,1995) is considered the most informative available experimental study on non-neoplastic effects (ASTDR, 2008). Effects comprised a decrease in body weight at the highest dose and an increased incidence of forestomach hyperplasia (indicative of chronic irritation) at 12.5 and 25 mg/kg/day. A NOAEL for non-carcinogenic effects was 2.5 mg/kg/day was proposed.

4.1.3 Carcinogenicity and mutagenicity

No data regarding the genotoxicity of 1, 3-dichloropropene to humans was identified. However, there have been a small number of case reports of the development of lymphomas or leukaemia as a result of acute and repeated exposure to 1, 3-dichloropropene (Markovitz & Crosby, 1984). However, the compound has not been classified as an established human carcinogene by an authoritive body to date.

Experimentally, in the two year dietary study in F344 rats reported above (Stott *et al.,* 1995), no increase in the incidence of malignancies was noted although the numbers of hepatocellular adenoma were higher than controls in males at 25 mg/kg/day. A NOAEL based on appearance of this tumour of 12.5 mg/kg/day was proposed.

Several *in vitro* assays have demonstrated the mutagenic activity of 1, 3-dichlorpropene in the presence of metabolic activation (EPA, 2000), however, ATSDR (2008) states that there is no conclusive experimental evidence of genotoxicity (ATSDR, 2008).

4.1.4 Reproductive and development toxicity

No information on the reproductive or developmental toxicity of 1, 3-dichloropropene in humans was identified.

Toxicokinetic studies carried out in animals have shown that 1, 3-dichloropropene or its metabolites are distributed to the reproductive organs (Waechter & Kastl, 1988).

In an inhalation study, F344 rats and New Zealand White rabbits were exposed to up to 120 ppm of 1, 3-dichlorpropene for six hours/day during gestation (days 6 – 15 and 6 – 18 in rats and rabbits respectively; Hanley *et al.,* 1988). Evidence of maternal toxicity was noted (decreased body weight) in both rats and rabbits at 300 ppm, but no effect on reproductive parameters was seen and developmental parameters were unaffected; a developmental NOAEL of 490 mg/m^3 was proposed.

No information on the reproductive or developmental toxicity of 1, 3-dichloropropene in humans was identified.

4.2 cis-3-chloroprop-2-enoic acid

4.2.1 Acute Toxicity

No information on the acute toxic effects of cis-3-chloroprop-2-enoic acid in humans was identified.

4.2.2 Repeat dose toxicity

No information on the repeat dose toxicity of cis-3-chloroprop-2-enoic acid in humans or animals to was identified.

Searches were made for publicly available information on the toxicity of cis-3-chloroprop-2-enoic acid, including use of the online programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.

Additional information was, therefore sought through use of the predictive 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK identified the presence of several structural alerts indicative of a number of possible toxicities. The following conclusions were drawn:

It is considered plausible that cis-3-chloroprop-2-enoic acid will exhibit carcinogenicity in humans. This endpoint is predicted because cis-3-chloroprop-2-enoic acid is a halogenated alkene compound

It is considered plausible that cis-3-chloroprop-2-enoic acid will cause hepatotoxicity in humans. This endpoint is predicted because cis-3-chloroprop-2-enoic acid is a halogenated hydrocarbon compound

It is considered plausible that cis-3-chloroprop-2-enoic acid will exhibit nephrotoxicity in humans. This endpoint is predicted because trans-3-chloroprop-2-enoic acid is a halogenated alkene compound.

The analysis by TOPKAT suggested that cis-3-chloroprop-2-enoic acid was:

Non-carcinogenic.

Assessment of mutagenicity and reproductive and developmental toxicity using TOPKAT was not possible for cis-3-chloroprop-2-enoic acid.

Table 1: Predicted toxicity data for cis-3-chloroprop-2-enoic acid using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H				
1,3-dichloropropene (experimental data)	130	2.7	NOAEL: 2.5 mg/kg/day Main target organ: Respiratory system	No evidence	Hepatocellular ademonas in rats	Negative
cis-3-chloroprop-2-enoic acid (TOPKAT)	1300 (188.8-8600)	272.1 (10-7400)	MTD (feed/drink) 21.5 mg/kg MTD (oral gavage) 59.3 mg/kg	Indeterminate	Negative	Indeterminate
cis-3-chloroprop-2-enoic acid (DEREK)	n/a	n/a	*Plausible* for hepatotoxicity and nephrotoxicity in humans	*Open* in humans	*Plausible* in humans	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; N/A – prediction not applicable to software

5 Guidelines and Standards

5.1 1, 3-dichloropropene

The EC risk classification is: T-toxic: R24/25; Xn-Harmful: R20, R65; Xi-Irritant: R36/37/38, 43; H-Handling: R10; N-Dangerous for the environment: R50, R53. 1, 3-dichloropropene is classified by the US EPA as 'slightly toxic'; it has not been classified by WHO because it is a fumigant. The International Agency for Research on Cancer Working Group (IARC, 1999) has concluded that there is *sufficient evidence in animals* to determine that 1,3-dichloropropene is *possibly carcinogenic in humans* (Group 2B). The US EPA has characterised the pesticide as Class B2 ie. probable human carcinogen (inadequate data in humans, sufficient data in animals; US EPA, 1996).

An ADI of 0.0125 mg/kg bw/day has been established (ATSDR, 2008) for 1,3-dichloropropene, using a safety factor (SF) of 200, based on a NOAEL of 2.5 mg/kg/day in a 2 year dietary study in which F344 rats were administered Telone II at up to 25 mg/kg/day (Stott *et al*., 1995; Lewis *et al*., 2007).

5.2 cis-3-chloroprop-2-enoic acid

Due to the lack of available human or experimental data, it has not been possible to establish a robust NOEL or NOAEL for cis-3-chloroprop-2-enoic acid. In addition, no ADI has been proposed for this metabolite by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The predicted toxicity profile for cis-3-chloroprop-2-enoic acid suggests that it may be less active than the parent. The highest oral MTD predicted by TOPKAT for cis-3-chloroprop-2-enoic acid is 59.3 mg/kg bw (gavage administration). This is significantly higher than the NOAEL of 2.5 mg/kg bw/day established for the parent compound.

Applying a SF of 1000 is recommended due to the possible carcinogenicity predicted by DEREK, giving a nominal value of 0.059 mg/kg bw/day. This is above the established ADI of 0.0125 mg/kg bw/day for the parent 1,3-dichloropropene. Therefore, – on a highly precautionary basis – it is proposed to adopt a PSDV of 0.0125 mg/kg bw/d for cis-3-chloroprop-2-enoic acid (i.e. the same value as for the parent); this will provide an overall SF of 5000.

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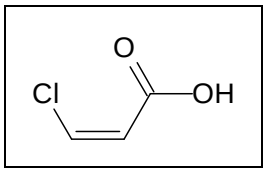
Appendix 12.6 trans-3-chloroprop-2-enoic acid

1 Introduction

Trans-3-chloroprop-2-enoic acid is a major metabolite of the nematicide 1,3 dichloropropene (CAS No. 542-75-6) that is formed within the plant, surrounding soil and groundwater. This metabolite is also formed through metabolism of 1,3-dichloropropene in humans.

The structure of trans-3-chloroprop-2-enoic acid is presented in Figure 1.

Figure 1: Structure of trans-3-chloroprop-2-enoic acid



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of 1,3-dichloropropene

1,3-dichloropropene is a general biocide comprised of cis- and trans-isomers. It is used primarily as a nematicide but also against certain plant diseases and in the control of insects and weeds on potatoes, tomatoes, tobacco, pineapple, and other vegetable and orchard crops. Typical usage rates are up to 280 kg/ha (Lewis *et al,* 2007).

1,3-dichloropropene is currently registered for use in the EU, Asia, South Africa, Pacific Region and North America under several commercial names including: Telone(R)Dowfume N; Telone C-17(R)Vidden D; Telone II(R)Terr-O-Cide 15-D; Telone II(R) bTerr-O-Cide 30-D; DD(R)Terr-O-Gas 57/43T; DD-92(R)Vorlex; M-3993 D-D 95; Dedisol C Nematox II; and Telone 2000 (IPCS, 1997). In general it is formulated as a colourless liquid and applied to the soil via drip irrigation systems or soil injection prior to planting (Lewis *et al*., 2007).

On 20th September 2007 the European Commission published a decision to withdraw existing authorisations for plant protection products containing 1,3-dichloropropene by March 2008, with no new authorisations subsequently being granted. A period of grace expiring no later than March 2009 was allowed (EC, 2007). A number of areas of concern were identified, particularly with regards to the release of unknown polychlorinated impurities into the environment, for which information on persistency, toxicology, uptake from crops, accumulation and metabolic fate was not available.

2.2 Environmental fate

2.2.1 Parent

1,3-dichloropropene is highly soluble in water (2485 mg/L at 20^{o}C, Lewis *et al*., 2007). In assessing the sorption to soil, a K_{oc} value of 34 L kg^{-1} has been described (Lewis *et al*., 2007), suggesting that the pesticide will be mobile in the environment.

The volatility of 1,3-dichloropropene is high and approximately 5 to 75% will volatise within 36 hr after soil injection. Degradation of 1,3-dichloropropene in the atmosphere occurs as a result of reactions with hydroxyl radicals, with a half-life of between 1 and 50 hr. It is not considered to be persistent in the atmosphere (US EPA, 2000).

1,3-dichloropropene is relatively rapidly degraded in soil with a degradation half-life of between 4.6 and 13.5 days under laboratory conditions (at 20ºC) and between 1 and 7 days under field conditions (IPCS, 1997).

2.2.2 Metabolite: trans-3-chloroprop-2-enoic acid
No information on the physiochemical properties or environmental fate of trans-3-chloroprop-2-enoic acid was identified.

2.3 Potential routes of human exposure
Potential routes of human exposure to 1,3-dichloropropene include inhalation, ingestion of contaminated drinking water and through dermal contact. The pesticide is usually not present in food as it has a short environmental persistence.

High levels of exposure to 1,3-dichloropropene are most likely to occur in occupational settings, either during production or use of the pesticide. Inhalation or dermal contact are the most probable routes of exposure in the workplace (ATSDR, 2008).

Although no data on routes of exposure of humans to the metabolite cis-3-chloroprop-2-enoic acid was identified, it is plausible that exposure may occur as a result of its ingestion in food or water contaminated from the breakdown of dichloropropene, or when formed as a human metabolite from ingestion of the parent compound.

3 Toxicokinetics
There are limited data with which to assess absorption following exposure of humans to 1,3-dichloropropene. Although no studies were identified relating to the oral absorption of 1,3-dichloropropene in humans, in one study, six male volunteers exposed to 1 ppm Telone II via inhalation for six hours showed between 72 and 80% absorption of the cis-isomer of 1,3-dichloropropene (ATSDR, 2008).

Dermal absorption was assessed through exposure of forearm skin in volunteers to cis-1,3-dichloropropene vapor at a concentration of 86 mg/m^3 (19 ppm) for 45 minutes. Recovery in urine (assessed by presence of the metabolite cis-1,3-dichloropropene-mercapturic acid) over a 20 hr period indicated between 2 - 5% absorption (Kezic *et al*., 1996).

Animal studies of the absorption of 1,3-dichloropropene following inhalation support findings in humans. In rats exposed to vapour concentrations of up to 900 ppm, absorption ranged from 82% to 62% (low to high exposures; Stott & Kastl, 1986). 1,3-dichloropropene was also easily absorbed following gavage dosing of ^{14}[C]-cis/ trans-1,3-dichloropropene in rats, with recovery of radioactivity in urine over a 24 hr period amounting to 82 - 84% (Climie et al., 1979). No studies were found relating to the absorption of 1,3-dichloropropene following dermal exposure in animals.

The distribution of 1,3-dichloropropene in humans following its inhalation at 1 ppm for 6 hr was assessed in six volunteers. Blood concentrations reached an apparent plateau within one hr of exposure, with levels of the cis-isomer ranging from 0.3 – 2 ppb (Waechter et al., 1992).

Experimentally, administration of ^{14}[C]-cis/trans-1,3-dichloropropene to rats by oral gavage resulted in a uniform concentration of radioactivity in most organs and tissues after 48 hr (Waechter & Kastl, 1988). The highest concentration of radioactivity was in the nonglandular stomach and the urinary bladder. No studies relating to the

distribution of any isomer of dichloropropene after dermal exposure in humans or animals were found.

1,3-dichloropropene is extensively metabolised although the rate of metabolism differs for the two isomers (Stott & Kastl, 1986). The metabolic pathway is common for all routes of exposure, and involves the rapid conjugation of 1,3-dichloropropene with glutathione in the liver, forming a mercapturic acid metabolite that is subsequently excreted via the kidney. Minor alternative pathways involve hydrolysis and dechlorination of the parent to form 1-chloroallyl alcohol or reaction with cytochrome P450 to form the mutagenic cis- and trans-epoxides that convert to 3-chloro2- hydroxy-propanal which is itself mutagenic (Schneider *et al*., 1998). ATSDR (2008) states that metabolic pathways are similar in rats and humans.

As noted above, the main excretory route is via urine. No studies relating to excretion of 1,3-dichloropropene following oral exposure in humans were identified. However, conjugates of cis-1,3-dichloropropene have been detected in the urine of four men occupationally exposed to Telone II®a at levels that correlate with exposure (Osterloh *et al*., 1984). The mercapturic acid metabolite of cis-1,3-dichloropropene was also detected in the urine of volunteers following dermal exposure (forearm skin) to a vapour concentration of 86 mg/m^3 (19 ppm) for 45 minutes (Kezic *et al*., 1996). In male volunteers exposed by inhalation to 1 ppm 1,3-dichloropropene for 6 hr, levels of parent compound plateaued in exhaled air by one hr and were no longer detectable one hr following the end of exposure. Urinary levels of N-acetyl-cysteine showed a biphasic pattern with half-lives of 4.2 - 3.2 hr during the initial phase and 12.3 - 17.1 hr during the second phase (Waechter *et al.,* 1992). The half-life for urinary excretion of the mercapturic acid metabolite following dermal exposure is reported to be approximately 6 hr (Kezic *et al.,* 1996).

Experimentally, a bi-phasic excretion has also been shown in male Fischer rats following inhalation exposure to 1,3-dichloropropene at 30, 90, 300 or 900 ppm for 3 hr. At doses up to 300 ppm, the half-life for the initial phase was 3 - 6 minutes, while that for the second phase was 33 - 43 minutes. At the highest dose, the initial half-life extended to 14 - 27 minutes (Stott & Kastl, 1986). The pattern of clearance following oral dosing of rats with 1,3-dichloropropene was reported to be similar (Stott *et al*., 1998).

No studies relating to excretion of 1,3-dichloropropene after dermal exposure in animals were identified.

No information on the toxicokinetics of trans-3-chloroprop-2-enoic acid was identified.

4 Toxicity Profile

4.1 1,3-dichloropropene

4.1.1 Acute Toxicity

Acute exposure of humans to 1,3-dichloropropene through inhalation has been anecdotally associated with a range of effects, most importantly the development of non-Hodgkin's (histiocytic) lymphoma and acute myelomonocytic anaemia (Markovitz &Crosby 1984), as well as a range of non-specific symptoms such as headache, nausea, vomiting, fatigue, impotence and malaise (Flessel *et al*., 1978; Markovitz & Crosby, 1984). The most common findings following inhalation exposure are chest discomfort, breathing difficulties, coughing and mucous membrane irritation. Concentrations of 1,3-dichlorpropene in excess of 1000 ppm may also cause lacrimation (ACGIH, 1989).

Ingestion of 1,3-dichloropropene has been reported to cause death with gastrointestinal, respiratory and cardiac effects preceding a multi-organ failure; at the time of death levels of 1,3-dichloropropene in blood and urine were 1.13 µmol/L and 0.20 µmol/L respectively (Hernandez *et al*., 1994). Delayed-type hypersensitivity reactions have also been reported in humans following dermal exposure (ATSDR, 2008).

Experimentally, 1,3-dichloropropene is moderately to highly toxic in rats; LD_{50}'s are 130 mg/kg, 333 mg/kg and 2.7 mg/m^3 by the oral, dermal and inhalation routes respectively (Lewis *et al*., 2007).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of 1,3-dichloropropene in humans was identified.

A dietary study in which F344 rats were administered Telone II at up to 25 mg/kg/ bw/day for two years (Stott *et al*.,1995) is considered the most informative available experimental study on non-neoplastic effects (ASTDR, 2008). Effects comprised a decrease in body weight at the highest dose and an increased incidence of forestomach hyperplasia (indicative of chronic irritation) at 12.5 and 25 mg/kg/day. A NOAEL for non-carcinogenic effects was 2.5 mg/kg/day was proposed.

4.1.3 Carcinogenicity and mutagenicity

No data regarding the genotoxicity of 1,3-dichloropropene to humans was identified. However, there have been a small number of case reports of the development of lymphomas or leukaemia as a result of acute and repeated exposure to 1,3-dichloropropene (Markovitz & Crosby, 1984). However, the compound has not been classified as an established human carcinogen by an authoritive body to date.

Experimentally, in the two year dietary study in F344 rats reported above (Stott *et al.,* 1995), no increase in the incidence of malignancies was noted although the numbers of hepatocellular adenoma were higher than controls in males at 25 mg/kg/day. A NOAEL based on appearance of this tumour of 12.5 mg/kg/day was proposed.

Several in vitro assays have demonstrated the mutagenic activity of 1,3-dichlorpropene in the presence of metabolic activation (EPA, 2000), however, ATSDR (2008) states that there is no conclusive experimental evidence of genotoxicity (ATSDR, 2008).

4.1.4 Reproductive and development toxicity

No information on the reproductive or developmental toxicity of 1,3-dichloropropene in humans was identified.

Toxicokinetic studies carried out in animals have shown that 1,3-dichloropropene or its metabolites are distributed to the reproductive organs (Waechter & Kastl, 1988).

In an inhalation study, F344 rats and New Zealand White rabbits were exposed to up to 120 ppm of 1,3-dichlorpropene for six hours/day during gestation (days 6 – 15 and 6 – 18 in rats and rabbits respectively; Hanley *et al.,* 1988). Evidence of maternal toxicity was noted (decreased body weight) in both rats and rabbits at 300 ppm, but no effect on reproductive parameters was seen and developmental parameters were unaffected; a developmental NOAEL of 490 mg/m^3 was proposed.

No information on the reproductive or developmental toxicity of 1,3-dichloropropene in humans was identified.

4.2 trans-3-chloroprop-2-enoic acid

4.2.1 Acute Toxicity

No information is available on the acute toxicity of trans-3-chloroprop-2-enoic acid to humans.

4.2.2 Repeat dose toxicity

No information of the repeat dose toxicity of trans-3-chloroprop-2-enoic acid in humans was identified.

Further searches were made for publicly available information on the toxicity of trans-3-chloroprop-2-enoic acid, including use of the online programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment programmes DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology) were utilised to inform on the potential properties of trans-3-chloroprop-2-enoic acid; results are summarised below (Table 1).

DEREK identified the presence of structural alerts indicative of a number of possible toxicities. The following conclusions were drawn:

It is considered plausible that trans-3-chloroprop-2-enoic acid will exhibit carcinogenicity in humans. This endpoint is predicted because trans-3-chloroprop-2-enoic acid is a halogenated alkene compound.

It is considered plausible that trans-3-chloroprop-2-enoic acid will cause hepatotoxicity in humans. This endpoint is predicted because trans-3-chloroprop-2-enoic acid is a halogenated hydrocarbon compound

It is considered plausible that trans-3-chloroprop-2-enoic acid will exhibit nephrotoxicity in humans. This endpoint is predicted because trans-3-chloroprop-2-enoic acid is a halogenated alkene compound

The analysis by TOPKAT suggested that trans-3-chloroprop-2-enoic acid was:

non-carcinogenic.

Assessment of mutagenicity and reproductive and developmental toxicity using TOPKAT was not possible for trans-3-chloroprop-2-enoic acid.

Table 1: Predicted toxicity data for trans-3-chloroprop-2-enoic acid using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
1,3-dichloro-propene (experimental data)	130	2.7	NOAEL: 2.5 mg/kg/day Main target organ: Respiratory system	No evidence	Hepatocellular ademonas in rats	Negative
trans-3-chloro-prop-2-enoic acid (TOPKAT)	1300 (188.8-8600)	272.1 (10-7400)	MTD (feed/drink) 21.5 mg/kg MTD (oral gavage) 59.3 mg/kg	Indeterminate	Negative	Indeterminate
trans-3-chloro-prop-2-enoic acid (DEREK)	n/a	n/a	*Plausible* for hepatotoxicity and nephrotoxicity in humans	*Open* in humans	*Plausible* in humans	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software

5 Guidelines and Standards

5.1 1,3-dichloropropene

The EC risk classification is: T-toxic: R24/25; Xn-Harmful: R20, R65; Xi-Irritant: R36/37/38, 43; H-Handling: R10; N-Dangerous for the environment: R50, R53. 1,3-dichloropropene is classified by the US EPA as 'slightly toxic'; it has not been classified by WHO because it is a fumigant. The International Agency for Research on Cancer Working Group (IARC, 1999) has concluded that there is *sufficient evidence in animals* to determine that 1,3-dichloropropene is *possibly carcinogenic in humans* (Group 2B). The US EPA has characterised the pesticide as Class B2 ie. probable human carcinogen (inadequate data in humans, sufficient data in animals; US EPA, 1996).

An ADI of 0.0125 mg/kg bw/day has been established (ATSDR, 2008) for 1,3-dichloropropene, using a safety factor (SF) of 200, based on a NOAEL of 2.5 mg/kg/day in a 2 year dietary study in which F344 rats were administered Telone II at up to 25 mg/kg/day (Stott *et al*., 1995; Lewis *et al*., 2007).

5.2 trans-3-chloroprop-2-enoic acid

Due to the lack of available human or experimental data, it has not been possible to establish a robust NOEL or NOAEL for trans-3-chloroprop-2-enoic acid. In addition, no ADI has been proposed for this metabolite by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The predicted toxicity profile for trans-3-chloroprop-2-enoic acid suggests that it may be less active than the parent. The highest oral MTD predicted by TOPKAT for trans-3-chloroprop-2-enoic acid is 59.3 mg/kg bw/day (gavage administration). This is significantly higher than the NOAEL of 2.5 mg/kg bw/day established for the parent compound.

Applying a SF of 1000 is recommended due to the possible carcinogenicity predicted by DEREK, giving a nominal value of 0.059 mg/kg bw/day. This is above the established ADI of 0.0125 mg/kg bw/day for the parent 1,3-dichloropropene. Therefore, – on a highly precautionary basis – it is proposed to adopt a PSDV of 0.0125 mg/kg bw/day for trans-3-chloroprop-2-enoic acid (i.e. the same value as for the parent); this will provide an overall SF of 5000.

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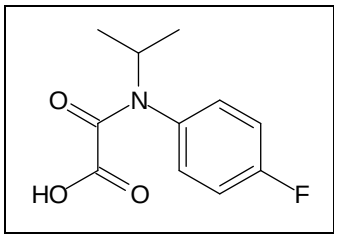
Appendix 12.7 FOE oxalate ([(4-fluorophenyl) (1-methylethyl)ammo]oxoacetic acid)

1 Introduction

FOE oxalate is a major metabolite of the herbicide flufenacet (CAS No. 142459-58-3). It is formed within the surrounding soil and may also be produced during the metabolism of absorbed flufenacet in humans and other organisms.

The structure of FOE Oxalate is presented in Figure 1.

Figure 1: Structure of FOE oxalate



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Flufenacet

Flufenacet is a selective herbicide used to control annual grasses and broadleaf weeds. It has a maximum usage rate of 0.14 kg flufenacet per hectare per year (US EPA, 1998). The pesticide is registered for use in the EU, Asia, South Africa, Pacific Region and North America under several commercial names including Artist, Cadu Star, Firebird, Shooter and Regatta (Lewis *et al*., 2007).

2.2 Environmental fate

2.2.1 Parent

Flufenacet is moderately soluble in water (56 mg/L at 20oC, Lewis et al., 2007).

A K_{oc} value of 202 L kg^{-1} has been described (Lewis *et al*., 2007) suggesting that the pesticide is moderately mobile in the environment.

If released into ambient air, flufenacet is likely to be present in both vapour and particulate phases. Vapour- phase flufenacet is degraded by reaction with photochemically-produced hydroxyl radicals with an estimated half-life of 22 hr. Particulate-phase flufenacet is removed by wet and dry deposition (HSDB, 2002)..
In soil, flufenacet is degraded principally by aerobic microbial action with a half-life of between 10 and 34 days (HSDB, 2002). The pesticide is considered to be moderately persistent in soil (Lewis *et al*., 2007).

2.2.2 Metabolite: FOE oxalate

FOE oxalate is one of the major metabolites formed from flufenacet, with an estimated formation fraction of 0.156 (15%; Lewis *et al*., 2007).

A K_{oc} value of 11 L kg^{-1} has been reported for FOE oxalate suggesting that the metabolite is very mobile in the environment (Lewis *et al.,* 2007).

In soil, FOE oxalate has a degradation half-life of 11 days and is not considered to be persistent (Lewis *et al.,* 2007).

No further information on the physicochemical properties or environmental fate of FOE oxalate was identified.

2.3 Potential routes of human exposure

High level exposure to flufenacet is most likely to occur in occupational settings either during production or use of the herbicide, with inhalation and dermal contact being the most probable routes. Exposure to flufenacet may also occur through ingestion of contaminated food or water or through by-stander exposure (HSDB, 2002).

Although no data on routes of exposure of humans to the metabolite FOE oxalate was identified, it is plausible that exposure may occur as a result of its ingestion in food or water contaminated from the breakdown of dichloropropene, or when formed as a human metabolite from ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of flufenacet in humans.

Experimental data are limited. In a rat metabolic study, radiolabeled flufenacet was rapidly absorbed and metabolised; 39 metabolites were identified. Urine was the major route of excretion with smaller amounts occurring in faeces (HSDB, 2002).

No information on the toxicokinetics of FOE oxalate was identified.

4 Toxicity Profile

4.1 Flufenacet

4.1.1 Acute Toxicity

No information is available on the acute toxicity of Flufenacet to humans.

Flufenacet has moderate acute toxicity in rats; LD_{50}'s are 598 mg/kg, 2000 mg/kg and 37.4 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis *et al*., 2007).

An acute rat neurotoxicity study has also reported decreased motor activity in males at doses of ≥75 mg/kg/day (US EPA, 1998).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of flufenacet in humans was identified.

In a dietary carcinogenicity study in rats, LOELs of 1.2 and 1.5 mg/kg/day in males and females were reported based on levels of methemoglobulinemia and multi-organ changes involving the blood, kidney, spleen, heart and uterus. A NOAEL was not proposed (US EPA, 1998).

4.1.3 Carcinogenicity and mutagenicity

No information on the carcinogenic or mutagenic potential of flufanacet in humans was identified.

In the repeat dose toxicity rat study described above, no evidence of carcinogenicity was found (US EPA, 1998).

Flufenacet was shown to be negative in gene mutation assays in bacterial and mammalian cells and in cytogenetic mammalian cell assays and a mouse micronucleus assay; flufenacet has no effect on unscheduled DNA synthesis (US EPA, 1998).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of flufenacet in humans was identified.

Studies in rats and rabbits have identified developmental effects at doses equal to or above those at which maternal toxicity occurred. A NOEL for maternal and developmental toxicity of 25 mg/kg bw/day was proposed in rats and 5 mg/kg bw/day in rabbits (US EPA, 1998).

4.2 FOE oxalate

4.2.1 Acute Toxicity

No information on the acute toxic effects of FOE oxalate in humans was identified.

No experimental studies on the acute toxic effects of FOE oxalate were identified.

4.2.2 Repeat dose toxicity

No information on the repeat dose toxicity of FOE oxalate in humans was identified.

Further searches were made for publicly available information on the toxicity of FOE oxalate, including use of the online programme ChemIDPlus (US NLM, 2003). However, no further information was identified.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1 Predicted toxicity data for FOE oxalate using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Flufenacet (experimental data)	598	3.74	LOEL: 1.2 mg/kg/day	Negative	Negative	Developmental effects in rats and rabbits
FOE Oxalate (TOPKAT)	265.4 (66.7-1100)	UE	MTD (feed/drink) UE MTD (oral gavage) UE	UE	Positive	Negative
FOE Oxalate (DEREK)	n/a	n/a	No alert	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software; UE-unrelaible estimate

DEREK did not identify any structural alerts for FOE oxalate.

The analysis by TOPKAT suggested that FOE oxalate was:

carcinogenic; and
not a developmental toxicant

Assessment of mutagenicity using TOPKAT was not possible for FOE oxalate.

5 Guidelines and Standards

5.1 Flufenacet

The EC risk classification is: Xn-Harmful: R22, R48/22; Xi-Irritant: R43; N-Dangerous for the environment: R50, R53. Flufenacet has a WHO classification of 'slightly hazardous' and a US EPA classification of 'slightly toxic' (Lewis *et al.,* 2007).

An ADI of 0.004 mg/kg bw/day has been proposed (EC, 2003) using a safety factor (SF) of 300 (10x for interspecies extrapolation, 10x for intraspecies variability, and 3x for the lack of a NOAEL), based on a repeat dose toxicity/carcinogenicity dietary study in rats (US EPA, 1998).

5.2 FOE oxalate

Due to the lack of available human or experimental data, it has not been possible to establish a robust NOEL or NOAEL for FOE oxalate. In addition, no ADI has been proposed for this metabolite by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The predicted toxicity profile for FOE oxalate is limited, with only comparison of LD_{50} being possible; however, based on this the metabolite appears to have similar activity to that of the parent. Therefore, – on a highly precautionary basis – it is proposed to adopt a PSDV of 0.004 mg/kg bow/day for FOE oxalate (i.e. the same value as for the parent).

6 References

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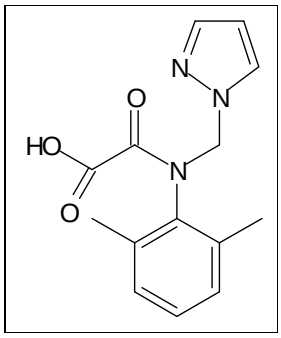
Appendix 12.8 Metazachlor oxalic acid (479M04)

1 Introduction

Metazachlor oxalic acid is a major metabolite of the herbicide metazachlor (2-chloro-*N*-(2,6-dimethylphenyl)-*N*-(1*H*-pyrazol-1-ylmethyl) acetamide); CAS No. 67129-08-2). It is formed by metabolism in the plant and by degradation in the surrounding soil and groundwater. It is also formed during metabolism of absorbed metazachlor in humans and other organisms.

The structure of metazachlor oxalic acid is presented in Figure 1.

Figure 1: Structure of metazachlor oxalic acid



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of metazachlor

Metazachlor is used as a selective herbicide for the control of annual grasses and broad-leaved weeds in a wide range of crops (artichokes, broccoli, asparagus, Brussels sprouts, cabbages, cauliflowers, sweet corn, garlic, horseradish, kale, leeks, maize, white mustard, onions, peanuts, pome fruits, potatoes, radish, rape, soya beans, stone fruits, strawberries, sugar cane, sunflowers, tobacco and turnips; Pesticide Manual, 1997).

Metazachlor is currently registered for use in around 40 countries in the EU, Africa and Asia and is marketed under several commercial names including Alpha Metazachlor, Nimbus CS, Rapasan 500SC, Shadow, Springbok and Sultan. In general, it is formulated as a suspension concentrate and applied as a spray (Lewis *et al*., 2007).

2.2 Environmental fate

2.2.1 Parent

Metazachlor is moderately soluble in water (450 mg/L at 20oC, Lewis et al., 2006). In assessing the sorption to soil, a K_{oc} value of 134 L kg^{-1} has been described (Lewis *et al*., 2007) suggesting moderate environmental mobility.

The volatility of metazachlor is low and therefore losses to the atmosphere from plant surfaces are expected to be small. Degradation of metazachlor in the atmosphere is relatively rapid; atmospheric degradation half-life is around 6.5 hr (EFSA, 2008) and it is therefore not considered to be persistent in the atmosphere.

Metazachlor is relatively rapidly degraded in soil with mean degradation half-lives of 10.8 days (at 20ºC) under laboratory conditions and 6.8 days under field conditions (EFSA, 2008).

2.2.2 Metabolite: Metazachlor oxalic acid

Metazachlor oxalic acid is one of the major metabolites formed during the metabolism or degradation of metazachlor, with an estimated formation fraction of 0.216 (approximately 20%; Lewis *et al*., 2007).

A K_{oc} value of 48 L kg^{-1} has been reported for metazachlor oxalic acid (Lewis *et al*., 2007) suggesting that the metabolite is mobile in the environment.

The degradation half-life of metazachlor oxalic acid in soil under laboratory conditions was 89.9 days. Under field conditions, the geometric mean DT_{50} at 20ºC was 56.4 days (EFSA, 2008). The metabolite is considered to be persistent (Lewis *et al.,* 2007).

Metazachlor oxalic acid is predicted to have high leachability, with a GUS leaching potential index of 3.53 and the SCI-GROW groundwater index (for a 1 kg ha^{-1} or 1 l ha^{-1} application) has been calculated as 6.51 X 10^{-01}µg L^{-1}(Lewis et al., 2007).

No further information on the physicochemical properties or environmental fate of metazachlor oxalic acid was identified.

2.3 Potential routes of human exposure

Exposure to metazachlor may occur through ingestion of contaminated food and water (oral route), or through occupational handling or by-stander exposure (inhalation or dermal contact).

Exposure of humans to the metabolite metazachlor oxalic acid may occur through ingestion of the metabolite in food and water contaminated with metazachlor and its metabolites or by metabolism of absorbed metazachlor.

Although no data on routes of exposure of humans to the metabolite cis-3-chloroprop-2-enoic acid was identified, it is plausible that exposure may occur as a result of its ingestion in food or water contaminated from the breakdown of dichloropropene, or when formed as a human metabolite from ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of metazacholor in humans.

Experimentally, CD rats were administered ^{14}[C]-metazachlor at 300 mg/kg bw by oral gavage as a single dose or on each of seven consecutive days. Following administration of the single dose, absorption was rapid with plasma radioactivity detectable within 15 min, and maximum levels being reached by 8 hr, for both sexes. However, radioactivity levels were approximately twice as high in females compared with males. Metazachlor was rapidly and widely distributed, with extensive metabolism occurring by a number of routes including hydroxylation of the pyrazole group, oxidation of the methylphenyl group, oxidative dechlorination, and glutathione and glucuronide conjugation. Excretion was predominantly via the urine (around 60 – 70%) in both sexes but was more rapid in females.

In the repeat dose study, six hours following administration of the final dose of ^{14}[C]-metazachlor, the highest level of radioactivity was associated with the GI tract. High levels were also maintained in whole blood; however, levels in plasma were much lower and showed a fall with time suggesting that binding of metazachlor or its

metabolites might be occurring. Binding to cellular components was attributed mainly to the liver and kidney. Although excretion was essentially complete within 144hr following administration of the final dose, relatively high levels of radioactivity were detected in the spleen, thyroid and lungs up to 10 days later (Hawkins *et al.,* 1980).

No information on the toxicokinetics of metazachlor oxalic acid was identified.

4 Toxicity Profile

4.1 Metazachlor

4.1.1 Acute Toxicity

No information is available on the acute toxicity of metazachlor in humans.

Experimentally, metazachlor has moderate acute toxicity in rats; LD_{50}'s are 2000 mg/kg, 2000 mg/kg and 34.5 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis *et al*., 2007).

Metazachlor is not a skin or eye irritant (EFSA, 2008) but there is evidence to suggest that it is a skin sensitiser (Gelbke & Grundler, 1980).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of metazachlor in humans was identified.

Experimentally, the principal target organ of metazachlor toxicity is the liver. Increased liver weights and enlarged, vacuolated hepatocytes were noted in male and female Sprague-Dawley rats given metazachlor via the diet at up to 6000 ppm for up to 113 weeks. At high doses, changes in clinical chemistry parameters were consistent with hepatotoxicity and haemolytic anaemia was also noted. No NOAEL was proposed (Hunter, 1983).

In a further study, Wistar rats were administered metazachlor in the diet at up to 8000 ppm for 2 years; bodyweight gain and food consumption were significantly reduced in males at 2000 ppm or above, and in females at 8000 ppm. In both sexes, clinical chemistry changes at high doses indicated a mild macrocytic anaemia, whilst at doses ≥ 200 ppm, an increase in total plasma bilirubin was evident. Mean and absolute liver and kidney weights were also increased at the highest dose. A NOAEL of 200 ppm (equivalent to 8.5 and 11.6 mg/kg bw/d in males and females respectively) was proposed based on a reduction in body weight gain and food consumption, and elevated plasma bilirubin levels (Krishnappa, 2002).

4.1.3 Carcinogenicity and mutagenicity

No information on the carcinogenicity and mutagenicity of metazachlor in humans was identified.

In the 2 year rat feeding study described above (Hunter, 1983), an increase in the incidence of thyroid parafollicular adenoma and thyroid parafollicular carcinoma was noted in male rats administered doses of 2000 or 6000 ppm metazachlor. Although a slightly higher incidence of hepatic adenoma were noted in male rats given 6000 ppm, this was not considered relevant to human risk assessment as this dose was also associated with hepatotoxicity in the rats (Hunter, 1983).

Metazachlor has been tested in a number of *in vitro* and *in vivo* genotoxicity studies. However, it has been concluded that metazachlor does not have genotoxic potential (EFSA, 2008).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of metazachlor in humans was identified.

In a two-generation reproduction study in which Crl:COBS CD(SD) rats were administered metazachlor via the diet at up to 1000 ppm for 15 weeks, no evidence reproductive toxicity was seen (Cozens, 1982). Parental and reproductive NOAELs of 1000 ppm were proposed (EFSA, 2008).

In a three-generation study in Wistar rats fed treated diet containing metazachlor at up to 8000 ppm, reduced litter sizes (secondary to reduced numbers of corpora lutea and implantations), together with reduced pup weight and post-natal survival, were seen at the high dose (Ganiger 1999). Parental and reproductive NOAELs of 151mg/kg bw/day and 192 mg/kg bw/day (2000 ppm) and a developmental NAOEL of 200 ppm (equivalent to 20 mg/kg bw/day) were proposed (EFSA, 2008).

No evidence of teratogenicity was seen in studies in the rat and rabbit, and a developmental NOAEL of 120 - 250 mg/kg bw/day in rabbits and 250 - 450 mg/kg bw/day in rats have been proposed (EFSA, 2008).

4.2 Metazachlor oxalic acid

Searches were made for publicly available information on the toxicity of metazachlor oxalic acid, including use of the online programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.

Additional information was, therefore, sought through use of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK identified the presence of one structural alert and the following conclusion was drawn:

It is considered plausible that metazachlor oxalic acid will exhibit skin sensitisation in humans. This endpoint is predicted because metazachlor oxalic acid is a formaldehyde donor.

The analysis by TOPKAT, while unable to derive an estimate of the oral MTD, suggested that metazachlor oxalic acid was:

non-carcinogenic;
non-mutagenic; but
a developmental toxicant.

Table 1 Predicted toxicity data for metazachlor oxalic acid using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Metazachlor (experimental data)	2000	34.5	NOAEL: 8.5 mg/kg/day Main target organ: Liver	No evidence	Thyroid parafollicular adenomas and carcinomas in rats	Reduced litter size, pup weight and survival (rats).
Metazachlor oxalic acid (TOPKAT)	UE	10,000 (10,000-10,000)	MTD (feed/drink) UE MTD (oral gavage) UE	Negative	Negative	Predicted developmental toxicant
Metazachlor oxalic acid (DEREK)	n/a	n/a	*Plausible* for skin sensitisation in humans	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software; UE – unreliable estimate

5 Guidelines and Standards

5.1 Metazachlor

The EC risk classification is: Xi – Irritant: R34; N – Dangerous for the environment: R50, R53. The EC safety classification is: S24, S37, S60, S61. Metazachlor is classified by WHO as 'unlikely to present acute hazard in normal use' while no consensus on classification was reached by the US EPA (Lewis *et al.,* 2007).

An ADI of 0.08 mg/kg bw/d has been proposed for metazachlor, with a safety factor (SF) of 100, based on a two year rat study in which Wistar rats received metazachlor via the diet at up to 8000 ppm (EFSA, 2008; Lewis *et al*., 2007). Although evidence of carcinogencity was noted in this study, a SF of 100 was adopted because this end point was not considered to be relevant to humans (Hunter, 1983).

5.2 Metazachlor oxalic acid

Due to the lack of availability of experimental data, it has not been possible to establish a robust NOAEL for metazachlor oxalic acid, and no ADI has been published by any authoritative organisation. In addition, no reliable estimate of predicted toxicity could be obtained from TOPKAT for metazachlor oxalic acid.

When compared with the parent, only the developmental toxicity is predicted to be carried forward by the metabolite. It is therefore, -on a highly precautionary basis – proposed to adopt a PSDV of 0.08 mg/kg bw/day for metazachlor oxalic acid (i.e. the same value as for the parent).

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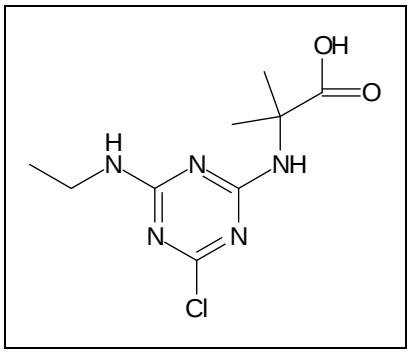
Appendix 12.9 Cyanazine chloroacid

1 Introduction

Cyanazine chloroacid is a major metabolite of the herbicide cyanazine (2-(4-cloro-6-ethylamino-1,3,5-triazin-2-ylamino)-2-methylpropiononitrile); CAS No. 21725-46-2). The metabolite is formed during plant metabolism and in surrounding soil; the metabolite is also formed through metabolism of absorbed cyanazine in humans.

The structure of cyanazine chloroacid, is presented in Figure 1.

Figure 1: Structure of cyanazine chloroacid



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of cyanazine

Cyanazine is a triazine herbicide currently sold under the trade names Bladex, DW3418, Fortrol, Match, and Payze for the control of annual grasses and broadleaf weeds; the pesticide does not currently have UK LERAP (local environment risk assessment for pesticides) approval. The pesticide is formulated and sold as a wettable powder, a flowable suspension or as granules (EXTOXNET, 1993). Although banned in the US, cyanazide is still in use in various African nations (e.g. South Africa, Niger), Asia and the Pacific Region (e.g. Australia, India, New Zealand and the Philippines), Europe (e.g., Hungary, Portugal and United Kingdom), Central Asia, Canada, and South America (Lynch, 2006).

2.2 Environmental fate

2.2.1 Parent

Use of cyanazine will result in its release into ambient air where the pesticide will be present in both vapour and particulate phases. Vapour-phase cyanazine will be degraded by reaction with photochemically-produced hydroxyl radicals; the estimated half-life of degradation is 41 hr (HSDB, 2001. Particulate-phase cyanazine will tend to be mainly removed through wet and dry deposition.

Cyanazine is moderately soluble in water (171 mg/L at 20^{o}C). If released into soil, the pesticide is expected to be moderately mobile, with a K_{oc} value of 190 L kg^{-1}. Degradation studies have shown cyanazine to be readily degraded in soils with a half-life under laboratory conditions (20^{o}C) of 16 days. It is not considered to be persistent (Lewis *et al*., 2007).

2.2.2 Metabolite: Cyanazine chloroacid

Cyanazine chloroacid is a major metabolite formed during metabolism or degradation of cyanazine.

No information on the physicochemical properties or environmental fate of cyanazine chloroacid was identified.

2.3 Potential routes of human exposure

Humans may be exposed to the parent cyanazine through ingestion of contaminated food or water or by inhalation or dermal contact during occupational handling or bystander exposure (HSDB, 1996).

Although no data on routes of exposure of humans to the metabolite cyanazine chloroacid was identified, it is plausible that exposure may occur as a result of its ingestion in food or water contaminated from the breakdown of cyanazine, or when formed as a human metabolite from ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of cyanazine in humans.

Toxicokinetic studies on cyanazine given at low doses to rats, dogs and cows via the diet indicate that it is rapidly absorbed from the GI tract (US EPA, 1988; full study details not available). Absorbed cyanazine has also been noted to accumulate in the brain, liver, kidney, muscle and fat. Cyanazine is rapidly metabolised and eliminated within four days in rats and dogs via the urine (40%) and faeces (47%; Hayes, 1991- full study details not available). In rats, the primary metabolic pathway that results in excretion in the urine involves N-de-ethylation to yield an amine; N-acetylcysteinyl derivatives are also found. The major metabolic route leading to faecal excretion involves de-chlorination to yield 2-hydroxy triazine (Hayes, 1991).

No information on the toxicokinetics of cyanazine chloroacid was identified.

4 Toxicity Profile

4.1 Cyanazine

4.1.1 Acute Toxicity

No information is available on the acute toxicity of cyanazine to humans.

In rats, cyanazine has moderate acute toxicity; LD_{50}'s are 182 mg/kg, 2000 mg/kg and 2.46 mg/m^3 for the oral, dermal and inhalation routes respectively (Lewis *et al*., 2007).

Cyanazine is considered to be a mild irritant to the eye (HSDB, 2001).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of cyanazine chloroacid in humans was identified.

In long-term feeding studies in rats and mice at up to 225 mg/kg bw/day, decreased body weight gain and increased liver weight was noted (Hayes, 1991; full study details not available). The principal target organ of toxicity for cyanazine has been reported to be the Central Nervous System (CNS; EXTOXNET, 1993).

4.1.3 Carcinogenicity and mutagenicity

No information on the carcinogenic and mutagenic toxicity of cyanazine in humans was identified.

Experimentally, cyanazine does not appear to be carcinogenic. In a mouse study at up to 50 mg/kg bw/day, no increase in the incidence of tumours was seen (Stevens, 1991; Hayes, 1991; full study details not available).

Conflicting findings have been reported in studies on the mutagenicity of cyanazine. DNA damage has been seen in mouse leukocytes *in vivo* at high doses of cyanazine (Tennant *et al.,* 2001; full study details not available). However, these changes were not repeated in studies on human lymphocytes and rat bone marrow (Hrelia *et al.,* 1994; full study details not available).

4.1.4 Reproductive and development toxicity

Although no detailed information on the reproductive and developmental toxicity of cyanazine in humans was identified, it is thought that reproductive effects are considered unlikely in humans exposed at environmental levels (HSDB, 2001).

In rats, cyanazine administered in the diet at 30 mg/kg bw/day resulted in decreased maternal body weight gain (US EPA, 1984; full study details unavailable). Maternal toxicity and decreased fetal viability was also reported for rabbits administered cyanazine in the diet at 2 mg/kg bw/day (US EPA, 1984; full study details unavailable).

In a multigeneration feeding study in rats administered 'moderate' doses of cyanazine, an increase in brain weight and decrease in kidney weight was noted in third generation offspring (Hayes, 1991; full study details unavailable). In female rats administered 'moderate' doses of cyanazine by oral gavage during pregnancy, decreased food intake of mothers and incomplete bone development of fetuses was noted. At higher doses, fetuses had cleft palates and absence, or underdevelopment, of eyes (Stevens, 1991; full study details unavailable). Birth defects were seen in offspring of exposed pregnant rats at doses as low as 1 mg/kg bw/day (HSDB, 2001). Other developmental abnormalities (including diaphragm and brain) have been noted in other treated species (HSDB, 2001).

4.2 Cyanazine chloroacid

Searches were made for publicly available information on the toxicity of cyanazine chloroacid, including use of the online programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1 : Predicted toxicity data for cyanazine chloroacid using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Cyanazine (experimental data)	182	2.46	NOEL 0.2 mg/kg/day Main target organ: CNS	No evidence	Negative	Decreased maternal body weight gain. Incomplete bone development, increase in incidence of cleft palate, underdeveloped eyeballs in foetus.
Cyanazine chloroacid (TOPKAT)	4800 (849-10,000)	10,000 (5800-10,000)	MTD (feed/drink) 64.5 mg/kg MTD (oral gavage) 178.5 mg/kg	Negative	Negative	Negative
Cyanazine chloroacid (DEREK)	n/a	n/a	*Plausible* that will exhibit hepatotoxicity, respiratory sensitisation and skin sensitisation in humans	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software

DEREK identified the presence of several structural alerts and the following conclusions were drawn:

It is considered plausible (i.e. there is a weight of evidence) that cyanazine chloroacid will exhibit hepatotoxicity in some humans. This endpoint is predicted because the compound is a 2,4-Diamino-1,2,5-triazine.

It is considered plausible (i.e. there is a weight of evidence) that cyanazine chloroacid will cause respiratory sensitisation in humans. This endpoint is predicted because the compound is a halo-diazine or -triazine.

It is considered plausible (i.e. there is a weight of evidence) that cyanazine chloroacid will exhibit skin sensitisation in humans. This endpoint is predicted because the compound is an activated N-heterocyclic .

The analysis by TOPKAT suggested that cyanazine chloroacid was:

non-carcinogenic;
non-mutagenic; and
not a developmental toxicant.

5 Guidelines and Standards

5.1 Cyanazine

The EC safety classification is: S2, S37, S60, S61. Cyanazine is classified by WHO as 'moderately hazardous', and by the US EPA as 'moderately toxic' (Lewis *et al.,* 2007).

An ADI of 0.002 mg/kg bw has been proposed for cyanazine by the Australian Government, using a safety factor (SF) of 100, based on based on long-term feeding study in which a NOEL of 0.2 mg/kg bw was derived (AODGP, 2008). However, due to the developmental toxicity seen in rabbits and rats at LOELs of 2 mg/kg bw/day and 1 mg/kg bw/day respectively, we also propose an ADI based on reproductive and developmental toxic effects, with a SF of 1000 applied. This would also give an ADI of 0.002 mg/kg bw (using LOEL form rabbit study).

5.2 Cyanazine chloroacid

Due to the lack of availability of experimental data, it has not been possible to establish a robust NOAEL for cyanazine chloroacid, and no ADI has been published by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

Overall, the toxicity profile for cyanazine chloroacid is predicted as somewhat less than that of the parent, with the highest oral MTD predicted by TOPKAT being 178.5 mg/kg bw/day (gavage). Applying a SF of 100 would give a nominal value of 1.78 mg/kg bw/day, which would be significantly above the proposed ADIs of 0.002 mg/kg bw/day for the parent cyanazine. Given this, it is – on a highly precautionary basis – therefore proposed to adopt a PSDV of 0.002 mg/kg bw/day for cyanazine chloroacid, i.e. the same value as for the parent; this will provide an overall SF of >10,000.

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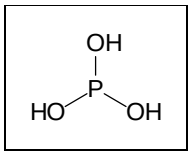
Appendix 12.10 Phosphorous Acid

1 Introduction

Phosphorous acid (CAS No. 13598-36-2) can be formed as a metabolite of the fungicide fosetyl-aluminium (aluminium tris-*O*-ethylphosphonate; CAS No. 39148-24-8) within the surrounding soil and plant. It is also formed during the metabolism of absorbed fosetyl-aluminium in humans. Phosphorous acid is also used as a fertiliser in its own right.

The structure of phosphorous acid is presented in Figure 1.

Figure 1: Structure of Phosphorous Acid



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of fosetyl-aluminium

Fosetyl-aluminium is a systematic fungicide used for both preventative and curative activities against Oomycetes, Alternaria and Pennicillium on avocado, cacao, citrus, hops, ornamentals, pineapple, rubber, strawberries, fruit crops, tobacco, vegetable crops and vines. It is also used to suppress bacterial pathogens such as fireblight (eawinia) on pome fruit, xanthomonas and on ornamentals (HSDB, 2006).

Fosetyl-aluminium is sold worldwide under the commercial names Aliette 80 WG and Fullstop; it is generally sold as wettable granules that are mixed with water and applied as a spray or used as a seed treatment (Lewis *et al*., 2007).

2.2 Environmental fate

2.2.1 Parent

Fosetyl-aluminium has high solubility in water (110,000 mg/L) and, in assessing the sorption to soil, a K_{oc} value of 1703 L kg^{-1} has been described suggesting slight mobility in the environment (Lewis *et al*., 2007).

Fosetyl-aluminium is highly susceptible to degradation in soil under aerobic conditions, with degradation half-lives of 0.1 and 0.04 days under laboratory ($20^{o}C$) and field conditions respectively; Fosetyl-aluminium is not considered to be persistent (Lewis *et al*., 2007).

2.2.2 Metabolite: Phosphorous acid

Phosphorous acid is one of the metabolites formed through degradation or metabolism of fosetyl-aluminium.

Phosphorous Acid has very high solubility in water (3100 g /L).
No further information on the physical properties or environmental fate of phosphorous acid was identified.

2.3 Potential routes of human exposure

Occupational exposure to fosetyl-aluminium may occur through inhalation and dermal contact with this compound at workplaces where the compound is produced or used (HSDB, 2006). The general population may be exposed to the fungicide via

dermal contact, inhalation of ambient air or through ingestion of drinking water or food contaminated with fosetyl-aluminium (HSDB, 2006).

Although no data on routes of exposure of humans to the metabolite phosphorous acid was identified, it is plausible that exposure may occur as a result of its ingestion in food or water contaminated from the breakdown of fosetyl-aluminium, or when formed as a human metabolite from ingestion of the parent compound.

3 Toxicokinetics
No information is available on the toxicokinetics of fosetyl-aluminium in humans.

In Sprague-Dawley rats given a single oral dose of radiolabeled fosetyl-aluminium at 3000 mg/kg bw, absorption was extensive and excretion rapid, being mainly complete within 24 hours of administration. Absorbed fosetyl-aluminium was distributed widely, with highest levels occurring in kidneys, liver, lungs, spleen, fat, adrenals, gonads and tissues with high metabolic activity. The main routes of excretion were through exhaled air (50%) and urine (32 – 33%), with faecal excretion accounting for 1.85% and 3.3% in males and females respectively, (EFSA, 2005).

Metabolism studies in rats have shown that fosetyl-aluminium **is** metabolised to phosphorous acid and ethanol; the latter is subsequently converted via acetaldehyde and acetate to carbon dioxide. The phosphorous acid produced is excreted largely unchanged, although limited oxidation to phosphate may also occur (ECB, 2000).
No information on the toxicokinetics of phosphorous acid was identified.

4 Toxicity Profile
4.1 Fosetyl-aluminium
4.1.1 Acute Toxicity
Fosetyl-aluminium is a severe eye irritant in humans (US EPA, 1990). No information on the acute toxicity of fosetyl-aluminium to humans was identified.

Experimentally, fosetyl-aluminium has low acute toxicity in rats; LD_{50}'s are 7080 mg/kg, 2000 mg/kg and 5.11 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis *et al*., 2007).

In animals, fosetyl-aluminium is non-irritating to the skin and no evidence of skin sensitisation has been reported (EFSA, 2005).

4.1.2 Repeat dose toxicity
No information on the repeat dose toxicity of fosetyl-aluminium in humans was identified.

Experimentally, in a two-year feeding study in Sprague-Dawley rats fed a diet containing fosetyl-aluminium at levels up to 30,000 ppm (equivalent, at the highest dose, to 1372 and 1786 mg/kg bw/day, for males and females respectively), no treatment-related effects on body weight, ophthalmology, haematology or clinical chemistry were reported (CEPA, 1998). A NOAEL of 348 and 450 mg/kg bw/day was proposed for males and females respectively.
Chronic exposure to fosetyl-aluminium was also assessed in Beagle dogs fed a diet containing up to 40,000 ppm (equivalent, for the high dose group, to 1228 and 1190 mg/kg bw/day for males and females respectively) for two years. No consistent treatment-related effects on body weight, food consumption, ophthalmology, haematology, clinical chemistry, or urinalysis were reported. Histopathogy showed increased incidence of seminiferous tubule degeneration in treated males consisting

of spermatocytic and/or spermatidic giant cells in the lumen of the seminiferous tubules; lesions were more numerous at 40,000 ppm than at 20,000 ppm, although the degree of severity was similar. In females, an increase in the incidence and severity of vacuolar tubular lesions of the kidney was reported with increasing dose. A NOEL of 10,000 ppm (348 and 450 mg/kg bw/day in males and females respectively) was proposed based on testicular effects in males (CEPA, 1998).

4.1.3 Carcinogenicity and genotoxicity

No information on the carcinogenic or mutagenic toxicity of fosetyl-aluminium in humans was identified.

In the chronic feeding study in rats described above, a dose-related increase in incidence of pheochromocytoma of the adrenal medulla was reported in males, with an associated decrease in incidence of focal hyperplasia; the effect on tumour incidence was considered treatment-related. In high dose males, urinary bladder neoplasia (carcinoma and papilloma combined) and transitional cell hyperplasia were also increased (CEPA, 1998).

The genotoxicity/ mutagenicity of fosetyl-aluminium has been assessed in a number of *in vivo* and *in vitro* assays; all assays have been negative (HSDB, 2006).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of fosetyl-aluminium in humans was identified.

In a three-generation reproductive toxicity study, CFY rats were fed diets containing fosetyl-aluminium at up to 24,000 ppm. At the highest dose, seven deaths were recorded in the F1B and three in the F2B generations; autopsy showed that these animals had haemorrhage of the urinary bladder wall, increased renal pelvic dilatation, interstitial nephritis and papillary necrosis. Body weight gain was reduced in both sexes at the highest dose, this was especially apparent in F1B and F2B generation males. Histopathology showed minimal epithelial hyperplasia and/or hypertrophy of the transitional epithelium of the urinary bladder, sometimes associated with small papillary projections and/or desquamation of epithelial cells in the lumen, for both sexes of F3B animals. No effects on reproductive parameters were seen and a reproductive NOEL of 24,000 ppm was proposedestablished (CEPA, 1998).

Developmental toxicity of fosetyl-aluminium was assessed in pregnant CFY rats by oral administration of up to 4000 mg/kg on days 6 to 15 of gestation. Maternal toxicity was apparent as reduced body weight gain and fetotoxicity as reduced viability of offspring. No adverse developmental effects were reported. A developmental NOEL of 1000 mg/kg was proposed (CEPA, 1998).

Developmental toxicity of fosetyl-aluminium was further assessed in pregnant New Zealand White rabbits subject to oral gavage at up to 500 mg/kg from day 6 to 16 of gestation. No developmental effects were reported (CEPA, 1998).

4.2 Phosphorous Acid

4.2.1 Acute Toxicity

No information on the acute effects of phosphorous acid in humans was identified.

Experimentally, Phosphorous Acid has low acute toxicity in rats; LD_{50}'s are 3624 mg/kg, 1650 mg/kg and > 6.14 mg/m^3 by the oral, dermal or inhalation routes respectively (EFSA, 2005).

Phosphorous Acid is an irritant to the eye but not the skin (EFSA, 2005).

4.2.2 Repeat dose toxicity

No information on the repeat dose toxicity of phosphorous acid in humans was identified.

Sub-chronic exposure to phosphorous acid has been assessed in a 90-day oral study in rats; a NOAEL of 400 mg/kg bw/day was established based on effects noted at 2000 mg/kg bw/day which included soft faeces and increased water intake and urinary sodium excretion (EFSA, 2005).

4.2.3 Carcinogenicity and genotoxicity

No information relating to carcinogenic or genotoxic potential in humans of phosphorous acid was identified.

In animals the potential carcinogenicity of phosphorous acid was assessed in a 117-week oral study in rats. No adverse effects were reported at the high dose and a carcinogenic NOAEL of 390 mg/kg bw/day was established. On this basis, EFSA considered that phosphorous acid is unlikely to pose a carcinogenic hazard to humans (EFSA, 2005).

Further searches were made for publicly available information of the toxicity of phosphorous acid, but no further toxicity data was identified. Additional insights were, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1: Predicted toxicity data for phosphorous acid using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$ (range)				
Fosetyl-aluminium (experimental data)	7080	5.11	NOAEL: 300 mg/kg/day (rat and dog)	Negative	Negative (humans)	Negative
Phosphorous acid (experimental data)	3624	> 6.14	400 mg/kg/day (rat)	Negative	Negative	n/d
Phosphorous acid (TOPKAT)	n/s	n/s	n/s	n/s	n/s	n/s
Phosphorous acid (DEREK)	n/a	n/a	No alert	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/s – not suitable for use with software; n/d – not determined; n/a – prediction not applicable to software;

DEREK identified no structural alerts in the molecule.

Phosphorous acid was unsuitable for analysis by TOPKAT.

5 Guidelines and Standards

5.1 Fosetyl-aluminium

The EC risk classification is: Xi - Irritant: R41. The EC Safety classification is S2, S26, S39, S46. Fosetyl-aluminium is classified by WHO as 'unlikely to present acute hazard in normal use' and by the US EPA as 'slightly toxic' (Lewis *et al*., 2007).

An ADI of 3 mg/kg bw/day has been established for fosetyl-aluminium using a safety factor (SF) of 100, based on two-year feeding studies in the rat and dog (EFSA, 2005).

5.2 Phosphorous acid

An ADI of 3.9 mg/kg bw/day has been established for phosphorous acid based on a two-year feeding study in rats with a safety factor (SF) of 100 applied (EFSA, 2005).

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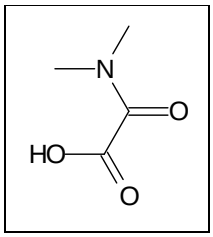
Appendix 12.11 Dimethyloxamic acid

1 Introduction

Dimethyloxamic acid is a major metabolite of the pesticide oxamyl (methyl N', N'-dimethyl-N-((methylcarbamoyl)oxy)-1-thiooxamimidate; CAS No. 23135-22-0). It is formed during plant metabolism and in the surrounding soil. It is also presumed that it may be formed during the metabolism of absorbed oxamyl by humans and other organisms (HSDB, 2004).

The structure of dimethyloxamic acid, is presented in Figure 1.

Figure 1: Structure of dimethyloxamic acid



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of oxamyl

Oxamyl is used as an insecticide, acaricide and nematicide on fruit, vegetable and field crops and ornamentals. It is commercially available as a water soluble liquid formula (24% a.i.), as a granule formulation (10% a.i.) and as technical material in cyclohexanone/water (42% a.i.). Typical application rates are: between soil furrows at 1-30 kg/ha; in soil dressing at 4.5-16.8 kg/ha; and, as a foliar spray at 0.4 kg/ha (IPCS, 1983). Oxamyl is sold under the trade names Blade, DPX 1410, Oxamil, Oxamimidic Acid, Pratt, Thioxamil, and Vydate (EXTOXNET, 1996).

2.2 Environmental fate

2.2.1 Parent

Use of oxamyl may result in release into the ambient air where it will exist as a vapour. Vapour-phase oxamyl will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals; the half-life of degradation is 1.4 days. Oxamyl is mobile in soil, with a K_{oc} value of 17 L kg^{-1}.

Studies have shown oxamyl to be readily degraded in soil, with half-life of 6.6 and 11 days, under laboratory and field conditions respectively (Lewis *et al*., 2007). The pesticide is not considered to be persistent.

2.2.2 Metabolite: Dimethyloxamic acid

Dimethyloxamic acid is one of the major metabolites formed following hydrolysis of Oxamyl, with an estimated maximum formation fraction of 0.395 (40%; Lewis *et al*., 2007).

A K_{oc} value of 6 L kg^{-1} has been reported for dimethyloxamic acid (Lewis *et al*., 2007) suggesting high mobility in the environment.

Studies have shown dimethyloxamic acid to be readily degraded in soil, with a half-life of 5 and 3.4 days under laboratory and field conditions respectively (Lewis *et al*., 2007). The metabolite is not considered to be persistent.

2.3 Potential routes of human exposure

Exposure of humans to the parent oxamyl may occur through ingestion of contaminated food or water or by inhalation or dermal contact during occupational handling or bystander exposure (HSDB, 2004).

Although no data on routes of exposure of humans to the metabolite dimethyloxamic acid was identified, it is plausible that exposure may occur as a result of its ingestion in food or water contaminated from the breakdown of oxamyl, or when formed as a human metabolite from ingestion of the parent compound.

3 Toxicokinetics

No information on the toxicokinetics of oxamyl in humans was identified.

In a single dose oral study, Sprague-Dawley rats were administered 1 mg/kg bw of [^{14}C]-oxamyl; following absorption, oxamyl distributed mainly to skin and muscle with less than 1% of administered dose being present in the liver, kidneys and brain. Approximately 80% of the dose was excreted in the urine within 24 hr. Following 168 hr after administration, 91% of the dose had been excreted in urine and 3% in faeces; the half-life of elimination of oxamyl was 3 - 8hr (HSDB, 2004).

In a further study, rats were administered 1mg (2.5 – 4.6 mg/kg bw) of [^{14}C]-oxamyl following an 18-day period in which non-radio labelled oxamyl was given in the diet at between 50 and 150 ppm (2.5 to 7.4 mg/kg bw/day). Radiolabelled oxamyl was found to be hydrolyzed to an oximino metabolite (methylN-hydroxy-N',N'-dimethyl-1-thioxamimidate) or converted enzymatically via N,N-dimethyl-1-cyanoformide (DMCF) to N,N-dimethyloxamic acid. Conjugates of the oximino compound, the acid, and their monomethyl derivatives constituted over 70% of the metabolites excreted in the urine and faeces (HSDB, 2004).

Based on experimental data, it therefore appears that metabolism of oxamyl may result in the production of an oxime and ketone. The dimethylacetyl group may hydrolyze to form N,N-dimethylformamid that would then be excreted as N-methylformamide and formamide in urine (Que Hee, 1993).

No information on the toxicokinetics of dimethyloxamic acid was identified.

4 Toxicity Profile

4.1 Oxamyl

4.1.1 Acute Toxicity

In humans, acute exposure to oxamyl can result in range of effects, including:

(a) Muscarinic effects: increased bronchial secretion, excessive sweating, salivation and lachrymation, excessive sweating, dizziness and headache pinpoint pupils, bronchoconstriction, abdominal cramps (vomiting and diarrhoea)and bradycardia;
(b) Nicotinic effects: involuntary contraction of fine muscles and tachycardia; and
(c) Central nervous system manifestations: headache, dizziness, anxiety, mental confusion, convulsions, coma, and depression of respiratory centre.
These symptoms may occur in different combinations and show variable onset times depending on the dose and route of exposure (HSDB, 2004). Ingestion of oxamyl (dose unknown) in an adult human was fatal within 12 hr (HSDB, 2004).

In rats, oxamyl has high acute toxicity; LD_{50}'s are 2.5 mg/kg, 2000 mg/kg and 0.056 mg/kg by the oral, dermal or inhalation routes, respectively (Lewis *et al*., 2007).

Oxamyl is considered a mild irritant to the eye and skin, and has slight to moderate sensitisation potential (Lewis *et al*., 2007).

4.1.2 Repeat dose toxicity

Repeat dose toxicity of oxamyl in humans may result in symptoms similar to those following acute exposure (EXTOXNET, 1996).

In rats fed diets containing up to 500 ppm oxamyl for two years, a decrease in body weight gain was noted at 100 and 150 ppm. Cholinesterase activity was lower at 150 ppm during the first seven days. All other endpoints considered were similar in control and treated groups. A NOEL of 50 ppm (2.5 mg/kg bw/day) was established, based on decreased body weight gain and food consumption (Kennedy, 1986).

In a further study, rats received oxamyl in the diet at levels up to 150 mg/kg bw for two years. A decrease in body weight change was seen after 4 weeks, which continued for the duration of the trial, with a dose-dependent decrease in body weight change at doses of 100 and 150 mg/kg bw. At 150 mg/kg bw, decreased cholinesterase activity was noted in females and males after four or eight days of treatment respectively; levels returned to normal by one month. Other adverse effects at the highest dose included: increased relative weights of brain, testes and adrenals in males, and brain, heart, lungs, kidney and adrenals in two female animals. No change in haematology, clinical chemistry or histopathology was observed at any dose (IPCS, 1983),

4.1.3 Carcinogenicity and mutagenicity

No information on the carcinogenic or mutagenic potential of oxamyl in humans was identified.

In the chronic toxicity /carcinogenicity study carried out in rats and described above (Kennedy, 1986) the type and incidence of tumours was unaffected by treatment; a NOEL of 50 ppm (2.5 mg/kg/day) was proposed for carcinogenic effects.

No evidence of mutagenicity has been found for oxamyl in either *in vitro* or *in vivo* assays (IPCS, 1983).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of oxamyl in humans was identified.

In a two-generation study in Crl:CDRBR rats were administered oxamyl in the diet at up to 150 ppm (11.6/15.8 mg/kg bw/day, in males and females respectively). At levels ≥ 75 ppm, offspring toxicity (decreased body weight gain during lactation) was noted and at 150 ppm, a decrease in number of live pups and reduced viability index was also apparent. A NOAEL of 75 ppm (5.2 and 6.6 mg/kg/day, for males and females respectively) was proposed for reproductive effects (HSDB, 2004).

No teratogenic or embryotoxic effects were noted in pregnant rats administered oxamyl in the diet (at 50, 100,150 or 300 mg/kg diet) during days 6 - 15 of gestation (IPCS, 1983).

4.2 Dimethyloxamic acid

Searches were made for publicly available information on the toxicity of dimethyloxamic acid, including use of the online programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK did not identify the presence of any structural alerts.

The analysis by TOPKAT suggested that dimethyloxamic acid was:

carcinogenic;
non-mutagenic; but
a developmental toxicant.

Table 1: Predicted toxicity data for dimethyloxamic acid using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$ (range)				
Oxamyl (experimental data)	2.5	0.056	NOAEL 2.5 mg/kg/day Main target systems: Muscarinic, Nicotinic, CNS	Negative	Negative	Reduced litter size and pup viability (rats).
Dimethyloxa-mic acid (TOPKAT)	1200 (226.8-6000)	6800 (396.1-10,000)	MTD (feed/drink) 113.4 mg/kg MTD (oral gavage) 313.3 mg/kg	Negative	Positive	Developmental toxicant
Dimethyloxa-mic acid (DEREK)	n/a	n/a	No alert	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software

5 Guidelines and Standards

5.1 Oxamyl

The EC risk classification is: T+-very toxic: R26/28; Xn-harmful: E21; N-dangerous for the environment: R51, R53. Ioxynil is also classified by WHO as 'highly hazardous' and by the US EPA as 'highly toxic' (Lewis *et al.,* 2007).

During a recent review of data on oxamyl, an ADI of 0.001 mg/kg bw/day has been proposed (EU, 2006; study details not defined).

5.2 Dimethyloxamic acid

Due to the lack of experimental data, it has not been possible to establish a robust NOEL or NOAEL for dimethyloxamic acid, and no ADI has been published by any

authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

Overall, the toxicity profile for dimethyloxamic acid is predicted as somewhat less than that of the parent, with the highest oral MTD predicted by TOPKAT being 313.3 mg/kg bw (by gavage administration). However, as the metabolite is also predicted to retain the developmental toxicity potential of the parent, and to have carcinogenic potential, it considered appropriate to apply a safety factor (SF) of 1000 which would give a nominal value of 0.313 mg/kg bw; this is significantly above the proposed SDI of 0.001 mg/kg bw/day for the parent oxamyl. Given this, it is – on a highly precautionary basis – therefore proposed to adopt a PSDV of 0.001 mg/kg bw/d for dimethyloxamic acid, i.e. the same value as for the parent; this will provide an overall SF >10,000.

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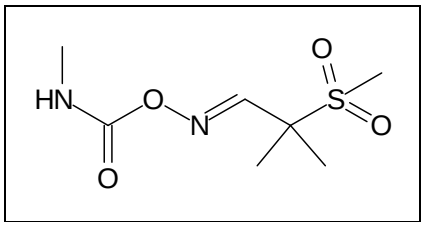
Appendix 12.12 Aldicarb sulfone (i.e. 2-methyl-2-(methylsulfonyl) propanal *o*-[(methylamino)carbonyl]oxime)

1 Introduction

Aldicarb sulfone (2-methyl-2-(methylsulfonyl)propanal *O*-[(methylamino)carbonyl]oxime; CAS No. 1646-88-4) is a major metabolite of the insecticide Aldicarb (2-methyl-2-(methylthio)propanal *O*-[(methylamino)carbonyl]oxime; CAS No. 116-06-3). It is formed within plants and surrounding soil and is also a metabolite formed during the metabolism of absorbed aldicarb by humans. Aldicarb sulfone also has application, in its own right, as an insecticide and nematicide.

The structure of aldicarb sulfone is presented in Figure 1.

Figure 1: Structure of aldicarb sulfone



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of aldicarb

Aldicarb is a systemic insecticide used to control mites, nematodes and aphids on cotton, peanut and soybean crops. Since 1990, it has been restricted to essential use only in the EU and is not used on potatoes due to concerns surrounding groundwater contamination. Aldicarb is currently sold under the commercial names Temik, ENT 27093, OMS 771, and UC 21149. The insecticide is formulated as a granular mix (10-15% a.i.) and applied directly to soil (EXTOXNET, 1996).

2.2 Environmental fate

2.2.1 Parent

Aldicarb has high water solubility (4930 mg/L at 20^{o}C). Studies assessing sorption to soil indicated a K_{oc} value of 30 L kg^{-1} suggesting mobility in the environment (Lewis *et al.,* 2007).

Degradation studies have shown aldicarb to be moderately persistent in soils with a degradation half-life of 2.4 days under laboratory conditions. The half-life of aldicarb in water has been reported to be between one day and a few months, and this pesticide is considered to be persistent. Degradation rates are considerably faster in surface water for which the half-life is between 5 and 10 days. Aldicarb is also metabolised by plants (EXTOXNET, 1996). Although the degradation of aldicarb in the atmosphere has not been well studied, this is not considered of importance since it is applied beneath the soil in a granular form (IPCS, 1991).

2.2.2 Metabolite: Aldicarb sulfone

Aldicarb sulfone is one of the major metabolites formed following metabolism or degradation of aldicarb. The estimated maximum formation fraction in soil is 0.2 (20%; Lewis *et al*., 2007).

Aldicarb sulfone is highly soluble in water (1000 mg/L at 20^{o}C) and is expected to be very mobile in the environment (K_{oc} value of 10 L kg^{-1}).

The half-life of degradation of aldicarb sulfone in soil is 20 or 21 days, under field and laboratory (20^{o}C) conditions respectively. This metabolite is also reported to be stable in water (Lewis *et al.,* 2007).

2.3 Potential routes of human exposure

Potential routes of exposure of humans to the parent compound aldicarb may arise through ingestion of contaminated food and water (oral route) or as a result of occupational handling where inhalation or dermal contact may occur (IPCS, 1991).

Although no data on routes of exposure of humans to the metabolite aldicarb sulfone was identified, it is plausible that exposure may occur as a result of its ingestion in food or water contaminated from the breakdown of aldicarb, or when formed as a human metabolite from ingestion of the parent compound (IPCS, 1991).

3 Toxicokinetics

No information is available on the toxicokinetics of aldicarb or aldicarb sulfone in humans.

Experimental studies have shown that aldicarb is absorbed almost completely from the GI tract. In one study, Andrawes *et al.* (1967) administered radio-labelled aldicarb to rats via the oral route and found 80 - 90% recovery of radioactivity in the urine within 24 hr. A further study also reported > 90% recovery of radio-labelled aldicarb in urine after oral administration to rats (Knaak *et al*., 1966).

In lactating Holstein cows administered radio-labelled aldicarb or aldicarb sulfone through oral doses of 0.006 to 0.52 mg/kg/day, 92% of the dose was recovered in urine over a 14 day period (Dorough *et al.,* 1970).

Dermal absorption of aldicarb in an aqueous solution or in toluene has been demonstrated qualitatively in rabbits (Kuhr & Dorough, 1976; Martin & Worthing, 1977) and rats (Gaines, 1969).

In a single dose oral study on female rats administered 0.4 mg/kg ^{35}[S]-aldicarb, showed that aldicarb and its metabolites were widely distributed throughout the body, with tissue showing preferential accumulation (Andrawes *et al.,* 1967). However, some accumulation of aldicarb and its metabolites was noted in the liver of cows after administration of up to 1.2 mg/kg diet of radio-labelled aldicarb in the diet for up to 14 days (Dorough *et al.,* 1970).

In laying hens given a single oral dose (0.7 mg/kg) of an equimolar mixture of aldicarb and aldicarb sulfone, accumulation of radiolabel was seen in liver and kidneys for the first 24 hr following administration; levels in fat and muscle were much lower (Hicks *et al.,* 1972).

Metabolic studies in rats have shown the formation of the sulfoxide, sulfone, oxime, sulfoxide, oxime sulfone, nitrile sulfoxide and nitrile sulfone from aldicarb. The sulfoxide and sulfone metabolites have also been shown to be formed in plants (Metcalf *et al*., 1966; Coppedge *et al*., 1967). Hydrolysis of aldicarb results in deactivation of its insecticidal activity; however, both the sulfoxide and sulfone metabolites are active anticholinesterase compounds in their own right (Andrawes *et al*., 1967; Bull *et al*., 1967; NAS, 1986).

As described previously, experimental studies have shown absorbed aldicarb to be rapidly metabolised and excreted principally in urine (Andrawes *et al.,* 1967; Knaak *et al*., 1966).

4 Toxicity Profile

4.1 Aldicarb

4.1.1 Acute Toxicity

Acute exposure of workers to aldicarb has been reported to cause symptoms of dizziness, blurred vision, constricted pupils, nausea and abdominal pain; depressed cholinesterase activity has also been reported (NRC, 1983). Very high doses can result in death due to paralysis of the respiratory system. Onset of symptoms occurs between 15 min and 3 hr following exposure and symptoms generally subside within 4 to 12 hr (HSDB, 2005). In rats, aldicarb has high toxicity; LD_{50}'s of 0.62 mg/kg, 20 mg/kg and 0.004 mg/m^3 have been reported for the oral, dermal and inhalation routes respectively (Lewis *et al.,* 2007).

Aldicarb is not an irritant to the eye or skin (Lewis *et al.,* 2007).

4.1.2 Repeat dose toxicity

A small number of human volunteer studies show that the principal target organ following repeat exposures to aldicarb is the nervous system. In a double-blind placebo-controlled study, 39 men were exposed to aldicarb via their diet at up to 0.075 mg/kg and nine women were given up to 0.05 mg/kg; of the subjects, six women and five men received a dose and a placebo exposure. At all doses, aldicarb treatment resulted in significant inhibition of red blood cell and plasma cholinesterase activity in both males and females. In males, reported clinical signs included sweating, light-headedness, headaches, and salivation and blood pressure changes. Peak effects occurred one hour after administration and the degree and duration of effect were dose-related. In contrast, no clinical signs or symptoms consistent with cholinesterase inhibition were noted in female volunteers. A NOAEL of 0.025 mg/kg bw/day was established based cholinesterase depression (Rhone-Poulenc, 1992; unpublished company report).

A two year feeding study in rats administered aldicarb at up to 0.1 mg/kg bw/day, assessed a number of parameters including, food consumption, mortality (and lifespan), incidence of infection, liver and kidney weight (as percentage of body weight), body weight gain, haematology, incidence of neoplasms, incidence of pathological lesions and brain, and plasma and erythrocyte cholinesterase levels. Animals receiving aldicarb were similar to controls for all parameters, and a NOAEL of ≥ 0.1 mg/kg bw/day was established for systemic toxicity (Union Carbide, 1966; unpublished company report – full study details not available).

4.1.3 Carcinogenicity and mutagenicity

No information on the carcinogenic or mutagenic potential of aldicarb in humans was identified.

Rats administered aldicarb at up to 0.1 mg/kg bw/day in the diet for two years did not show an increased incidence of neoplasms (Union Carbide, 1966; unpublished company report – full study details not available).
In a further study on Greenacres Laboratory (controlled flora) rats administered aldicarb via the diet at up to 0.3 mg/kg bw/day for two years, no increase in incidence of tumours was found. A NOAEL for carcinogenic effects of 0.3 mg/kg bw/day was proposed (Union Carbide, 1972; unpublished company report).

Aldicarb did not show geneotoxic activity in bacterial or mammalian gene mutation assays (PSD, 1994).

4.1.4 Reproductive and development toxicity

No information was found on the reproductive and developmental effects of aldicarb in humans.

In a three-generation reproduction study in rats fed aldicarb in the diet at up to 0.7 mg/kg day, histolopathological examination was undertaken on groups of the third generation (F3) animals at weaning and at 90 days of age. No changes in reproductive tissues were seen at any dose tested. However, F2 pups showed decreased body weight at birth at the highest dose tested (0.7 mg/kg/day) and a NOAEL of 0.3 mg/kg day for fetotoxicity was established (Union Carbide, 1974; unpublished company report).

In a developmental toxicity study, pregnant CD rats were administered aldicarb by gavage at up to 0.5 mg/kg bw/day. Decreased maternal body weight and food consumption were reported at 0.25 and 0.5 mg/kg bw/day and some maternal deaths occurred at the highest dose. In offspring, developmental effects were noted at 0.5 mg/kg bw/day; these included significantly increased dilation of lateral ventricles of the brain, poor ossification of the sixth sternebra, together with significantly decreased foetal body weight. At 0.25 and 0.5 mg/kg bw/day, significant increases in the incidence of small haemorrhages on the trunk were also noted. A NOEL for developmental effects of 0.125 mg/kg bw/day was proposed (Rhone-Poulenc, 1988; unpublished company report).

4.2 Aldicarb sulfone

4.2.1 Acute Toxicity

No information on the acute toxicity of aldicarb sulfone in humans was identified.

In rats, aldicarb sulfone has high toxicity; LD_{50}'s of 27 mg/kg, 20 mg/kg and 0.209 mg/m^3 have been reported for the oral, dermal or inhalation routes respectively (Lewis *et al.,* 2007).

Aldicarb sulfone is not an irritant to the eye or skin (Lewis *et al.,* 2007).

4.2.2 Repeat dose toxicity

No information on the repeat dose toxicity of aldicarb sulfone in humans was identified.

Experimentally, aldicarb sulfone was administered to rats in the diet as a 1:1 mixture with aldicarb sulfoxide at up to 1.2 mg/kg bw/day. An elevated mortality rate was noted during the first 30 days of treatment for high dose groups receiving the mixture. At the highest dose, reduced growth performance was apparent in males and a lower plasma cholinesterase activity in both males and females. No treatment-related effects were noted in lower dose groups and a NOEL of 2.4 mg/kg bw/day was proposed for the mixture (IPCS, 1991).

Experimentally, aldicarb sulfone was fed to Beagle dogs in the diet at up to 100 ppm (2.21mg/kg bw/day) for 1 year. At study termination, brain cholinesterase activity was noted to be significantly inhibited in males given the highest dose, and in females

receiving mid or high doses. Plasma cholinesterase activity was also significantly inhibited at all doses in males and at mid and high doses in females. Red blood cell cholinesterase activity was significantly inhibited in high-dose males and females and mid-dose females. A NOAEL of 5 ppm (0.11 mg/kg bw/day) was established, based on brain cholinesterase inhibition (Union Carbide, 1987; unpublished company report).

Additional information regarding the toxicity of aldicarb sulfone was also sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK identified the presence of only one structural alert and the following conclusion was drawn:

It is considered plausible (i.e. there is a weight of evidence) that aldicarb sulfone will exhibit cholinesterase inhibition in humans. This endpoint is predicted because aldicarb sulfone is an N-Methyl or N,N-dimethyl carbamate compound.

The analysis using TOPKAT suggested that aldicarb sulfone was:

a developmental toxicant

Predictions of the carcinogenicity and mutagenicity of aldicarb sulfone was not possible using TOPKAT.

Table 1: Predicted toxicity data for aldicarb sulfone using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m³/H (range)				
Aldicarb (experimental data)	0.62	0.004	NOAEL 0.025 mg/kg/day Main target: Nervous system	Negative	Negative	Developmental effects (rats)
Aldicarb sulfone (experimental data)	27	0.209	NOAEL 0.11 mg/kg/day Main target: Nervous system	NR	NR	NR
Aldicarb sulfone (TOPKAT)	67 (10.9-412.1)	370.1 (16-8600)	MTD (feed/drink) 4.9 mg/kg MTD (oral gavage) 13.5 mg/kg	UE	UE	Developmental toxicant
Aldicarb sulfone (DEREK)	n/a	n/a	*Plausible* that aldicarb sulfone will exhibit cholinesterase inhibition in humans	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; NR- not reported; MTD – maximum tolerated dose; n/a – prediction not applicable to software

5 Guidelines and Standards

5.1 Aldicarb

The EC risk classification is: T+-Very toxic: R26/28; T-Toxic: R24; N – Dangerous for the environment: R50, R53 (Lewis *et al*., 2007). Aldicarb is also classified by WHO as 'extremely hazardous', and by the US EPA as 'highly toxic' (Lewis *et al*., 2007).

An ADI of 0.003 mg/kg bw/day has most recently been established (WHO, 2003) for aldicarb, with a safety factor (SF) of 10, based on cholinesterase depression in a single oral dose study in humans.

5.2 Aldicarb sulfone

Aldicarb sulfone is classified by WHO as 'extremely hazardous', and by the US EPA as 'highly toxic'. No EC risk classifications have been established (Lewis *et al*., 2007).

An ADI of 0.001 mg/kg bw/day has been established (NRA, 2003) for aldicarb sulfone, with a SF of 100, based on based on brain cholinesterase inhibition in a one year feeding study in dogs.

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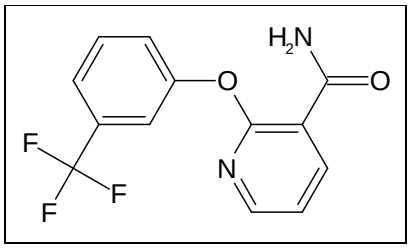
Appendix 12.13 AE 0542291 (2-(3-trifluoromethylphenoxy) nicotinamide)

1 Introduction

2-(3-Trifluoromethylphenoxy) nicotinamide, also known as AE 0542291 (CAS 36701-89-0) is a major metabolite of the herbicide Diflufenican (*N*-(2,4-difluorophenyl)-2-[3-(trifluoromethyl)phenoxy]-3-pyridinecarboxamide; CAS No. 83164-33-4). The metabolite is formed through degradation within soil and, to a small extent, during metabolism of absorbed diflufenican in humans and other organisms.

The structure of AE 0542291 is presented in Figure 1.

Figure 1: Structure of AE 0542291



2 Use, environmental fate of parent and potential human exposure routes

2.1 Use of diflufenican

Diflufenican is an anilide herbicide that is currently sold worldwide under the commercial names Absolute, Ardent, Bacara, Firebird, Graduate, Ingot, Javelin Gold, Hurricane SC, Othello, Pelican and Regatta. It is used in mixtures to control broad-leaved and grass weeds during early post-emergence in winter wheat and winter barley (PSD, 1994). Diflufenican is often sold as a soluble concentrate which is mixed with water and applied as a spray (Lewis *et al*., 2007).

2.2 Environmental fate

2.2.1 Parent

Diflufenican has low water solubility (0.05 mg/L at 20^{o}C). It has a K_{oc} value of 3186 L kg^{-1} suggesting slight mobility in the environment (Lewis *et al*., 2007).

Studies have shown that diflufenican is not readily degraded in soils; half-life in soil is 141.8 and 315 days under laboratory and field conditions respectively. It is considered persistent in the environment (Lewis *et al*., 2007; PSD, 1994).

2.2.2 Metabolite: AE 0542291

AE 0542291 is one of the major metabolites/degradation products of diflufenican, with an estimated maximum formation fraction of 0.263 (26%; Lewis *et al*., 2007).

AE 0542291 has moderate solubility in water (100 mg/L at 20^{o}C) and has a K_{oc} value of 132 L kg^{-1}, suggesting moderate mobility in the environment (Lewis *et al*., 2007).

Studies have shown that AE 0542291 is readily degraded in soils with an estimated half-life of 26.9 days under laboratory conditions (20^{o}C). It is not considered to be persistent in the environment (Lewis *et al*., 2007).

2.3 Potential routes of human exposure

Exposure of humans to the parent diflufenican may occur through ingestion of contaminated food, water or soil. Exposure through inhalation or dermal contact during occupational handling or by-stander exposure is also possible (PSD, 1994).

Although no data on routes of exposure of humans to the metabolite AE 0542291was identified, it is plausible that exposure may occur as a result of its ingestion in food or water contaminated from the breakdown of diflufenican, and to a small extent when formed as a human metabolite from ingestion of the parent compound (IPCS, 1991).

3 Toxicokinetics
No information is available on the toxicokinetics of diflufenican in humans.

In an oral dose study in male and female rats administered a single dose of radio-labelled diflufenican, 87 - 97% of the dose was excreted unchanged in the faeces. Renal excretion accounted for between 2 and 7% of the radioactivity; of this approximately 75% was the unchanged parent and four metabolites accounting for only between <0.1% and 4.4% of the administered dose. The metabolites were identified as products of hydroxylation and defluorination with hydroxyl-substitution of the diflurophenyl ring, removal of the difluorophenyl ring to generate free acid and probable hydroxylation of the (trifluoromethyl) phenyl ring (PSD, 1994).

No information on the toxicokinetics of AE 0542291 was identified.

4 Toxicity Profile
4.1 Diflufenican
4.1.1 Acute Toxicity
No information is available on the acute toxicity of diflufenican in humans.

In rats, diflufenican has low acute toxicity; LD_{50}'s are 5000 mg/kg, 2000 mg/kg and 5.12 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis *et al*., 2007).

Diflufenican is not an irritant to the eye or skin in rabbits, and no evidence of sensitisation was identified (PSD, 1994).

4.1.2 Repeat dose toxicity
No information on the repeat dose toxicity of diflufenican in humans was identified.

Experimentally, in F344 rats fed for two years on diets containing up to 12,500 ppm diflufenican (equivalent to 6250 mg/kg bw/day), reduced food consumption was noted in females at levels of 2500 ppm and above. Final body weights were also significantly lower in males at 500 ppm (by 7%) and in both sexes at ≥ 2500ppm (by 11 - 22%). A dose–related reduction in urinary volume, and increased urinary acidity and specific gravity were also noted, particularly in females. Organ weight effects included a significant increase in kidney weight in female at 12,500 ppm, a slight increase in lung weight in males at ≥ 2500 ppm, increased spleen weight in females at ≥ 2500 ppm and reduced thymus weight in males at ≥ 500 ppm. A NOAEL of > 500 ppm (25 mg/kg bw/day) was established based on reductions in body weight gain and thymus weight at 500 ppm diflufenican (PSD, 1994).

4.1.3 Carcinogenicity and mutagenicity
No information was found on the carcinogenic or mutagenic potential of diflufenican in humans was identified.
In the two year rat feeding study described above, diflufenican was not carcinogenic (PSD, 1994).

Diflufenican has been shown to be negative in a reverse mutation assay (in the presence or absence of metabolic activation), a chromosomal mutation assay (in the

presence or absence of metabolic activation), in an unscheduled DNA synthesis assay and an *in vivo* cytogenetics assay (PSD, 1994).

4.1.4 Reproductive and development toxicity

No information on the reproductive or developmental toxicity of diflufenican in humans was identified.

In a rat multigeneration study, reduced birth weight of pups and weight during lactation was reported at the highest administered dose in all generations (above 500 ppm). Reduced litter size, increased pup mortality and reduced thymus weight were reported in offspring of all generations receiving the highest dose. A NOEL of 500 ppm (25 mg/kg bw/day) was proposed for reproductive effects (PSD, 1994; full study details not available).

In a teratogenicity study in the rat, the minimum effect level for maternal toxicity was 50 mg/kg bw/day based on reductions in body weight gain at all doses. The NOEL for fetotoxicity and teratogenicity was 5000 mg/kg bw/day (PSD, 1994; full study details not available).

4.2 AE 0542291

Searches were made for publicly available information on the toxicity of AE 0542291, and by use of the online programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1: Predicted toxicity data for AE 0542291 using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$ (range)				
Diflufenican (experimental data)	5000	5.12	NOAEL:> 25 mg/kg/day. Main target organ: Thymus	Negative	Negative	Reduced pup weight (rats; all generations). Reduced litter size, increased pup mortality (rats; all generations).
AE 0542291 (TOPKAT)	79 (13-479.9)	10,000 (7200-10,000)	MTD (feed/drink) 5 mg/kg MTD (oral gavage) 13.8 mg/kg	UE	Positive	Developmental Toxicant
AE 0542291 (DEREK)	n/a	n/a	No alert	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software; UE-unreliable estimate

DEREK did not identify any structural alerts.

The analysis by TOPKAT suggested that AE 054229 was:

carcinogenic; and
a developmental toxicant.

Prediction of the mutagenic potential of AE 054229 using TOPKAT was not possible.

5 Guidelines and Standards

5.1 Diflufenican

The EC risk classification is: N – Dangerous for the environment: R50, R53, and EC safety classification is: S60, S61. Diflufenican is classified by WHO as 'Unlikely to present acute hazard in normal use (Lewis *et al*., 2007).

An ADI of 0.2 mg/kg bw/d has been established (PSD 1995; EFSA, 2007) for diflufenican using a safety factor (SF) of 100, based on the critical minimum effect level of 25 mg/kg bw/day derived from a two year dietary study or multigeneration reproductive study in rats.

5.2 AE 0542291

Due to the lack of availability of experimental data, it has not been possible to establish a robust NOEL or NOAEL for AE 0542291 and no ADI has been published by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The toxicity profile for AE 0542291 is predicted to be similar overall to that of the parent with the highest oral MTD predicted by TOPKAT estimated at 13.8 mg/kg bw (gavage administration). Applying a SF of 100 would give a nominal value of 0.138 mg/kg bw, which is similar to the established ADI of 0.2 mg/kg bw/day for the parent diflufenican. There is some discrepancy in predictions for carcinogenic potential and reproductive and developmental toxicity between DERK and TOPKAT; however, any concerns that this raises would be fully addressed in the SF used to establish the parental ADI. Given this, it is – on a highly precautionary basis – proposed to adopt a PSDV of 0.2 mg/kg bw/d for AE 0542291, i.e. the same as that of the parent.

6 References

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Lewis K, Green A, Tzilivakis J (2007) Pesticide database holding fate and ecotoxicological values. Report DL24 of the FP6 EU-funded FOOTPRINT project. Available at http://www.eu-footprint.org.

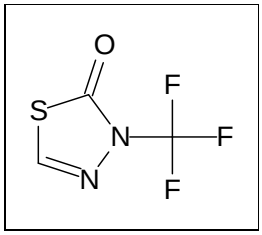
Pesticides Safety Directorate (Defra PSD, 1994) Evaluation on: Diflufenican. Available at: http://www.pesticides.gov.uk/PSD_PDFs/Evaluations/137_diflufenican.pdf

Appendix 12.14 Thiadone (3-trifluoromethyl-1,3,4-thiadiazol-2(3H)one)

1 Introduction

Thiadone (3-trifluoromethyl-1,3,4-thiadiazol-2(3H)one) is a metabolite of the herbicide flufenacet (CAS No. 142459-58-3). The structure of thiadone is presented in Figure 1.

Figure 1: Structure of thiadone



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of flufenacet

Flufenacet is a selective herbicide used to control annual grasses and broadleaf weeds. It has a maximum usage rate of 0.14 kg flufenacet per hectare per year (US EPA, 1998). The pesticide is registered for use in the EU, Asia, South Africa, Pacific Region and North America under several commercial names including Artist, Cadu Star, Firebird, Shooter and Regatta (Lewis *et al*., 2007).

2.2 Environmental fate

2.2.1 Parent

Flufenacet is moderately soluble in water (56 mg/L at 20^{o}C, Lewis *et al*., 2007).

A K_{oc} value of 202 L kg^{-1} has been described (Lewis *et al*., 2007) suggesting that the pesticide is moderately mobile in the environment.

If released into ambient air, flufenacet is likely to be present in both vapour and particulate phases. Vapour- phase flufenacet is degraded by reaction with photochemically-produced hydroxyl radicals with an estimated half-life of 22 hr. Particulate-phase flufenacet is removed by wet and dry deposition (HSDB, 2002)..

In soil, flufenacet is degraded principally by aerobic microbial action with a half-life of between 10 and 34 days (HSDB, 2002). The pesticide is considered to be moderately persistent in soil (Lewis *et al*., 2007).

2.2.2 Metabolite: Thiadone

Thiadone is one of the metabolites formed from flufenacet. Water sediment metabolism studies have shown thiadone is present at levels of up to 82% of the initial dose in water and <10% in sediment. The metabolite has not been identified in soil dissipation studies (limit of detection 0.01 mg/kg; EC, 2001).

Thiadone is not rapidly degraded and has been reported to persist at 80% of the initial dose after 55 days (EC, 2001). It is considered to be persistent in the environment.

No further information relating to the physicochemcical properties or environmental fate of thiadone was identified.

2.3 Potential routes of human exposure

High level exposure to flufenacet is most likely to occur in occupational settings either during production or use of the herbicide, with inhalation and dermal contact being the most probable routes. Exposure to flufenacet may also occur through ingestion of contaminated food or water or through by-stander exposure (HSDB, 2002).

Although no data on routes of exposure of humans to the metabolite FOE oxalate was identified, it is plausible that exposure may occur as a result of its ingestion in food or water contaminated from the breakdown of dichloropropene, or when formed as a human metabolite from ingestion of the parent compound.

3 Toxicokinetics
No information is available on the toxicokinetics of flufenacet in humans.

Experimental data are limited. In a rat metabolic study, radiolabeled flufenacet was rapidly absorbed and metabolised; 39 metabolites were identified. Urine was the major route of excretion with smaller amounts occurring in faeces (HSDB, 2002).

No information on the toxicokinetics of thiadone was identified.

4 Toxicity Profile
4.1 Flufenacet
4.1.1 Acute Toxicity
No information is available on the acute toxicity of flufenacet to humans.

Flufenacet has moderate acute toxicity in rats; LD_{50}'s are 598 mg/kg, 2000 mg/kg and 37.4 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis *et al*., 2007).

An acute rat neurotoxicity study has also reported decreased motor activity in males at doses of ≥75 mg/kg/day (US EPA, 1998).

4.1.2 Repeat dose toxicity
No information on the repeat dose toxicity of flufenacet in humans was identified.

In a dietary carcinogenicity study in rats, LOELs of 1.2 and 1.5 mg/kg/day in males and females were reported based on levels of methemoglobulinemia and multi-organ changes involving the blood, kidney, spleen, heart and uterus. A NOAEL was not proposed (US EPA, 1998).

4.1.3 Carcinogenicity and mutagenicity
No information on the carcinogenic or mutagenic potential of flufanacet in humans was identified.

In the repeat dose toxicity rat study described above, no evidence of carcinogenicity was found (US EPA, 1998).

Flufenacet was shown to be negative in gene mutation assays in bacterial and mammalian cells and in cytogenetic mammalian cell assays and a mouse micronucleus assay; flufenacet has no effect on unscheduled DNA synthesis (US EPA, 1998).

4.1.4 Reproductive and development toxicity
No information on the reproductive and developmental toxicity of flufenacet in humans was identified.

Studies in rats and rabbits have identified developmental effects at doses equal to or above those at which maternal toxicity occurred. A NOEL for maternal and developmental toxicity of 25 mg/kg bw/day was proposed in rats and 5 mg/kg bw/day in rabbits (US EPA, 1998).

4.2 Thiadone

4.2.1 Acute Toxicity

No information on the acute toxic effects of thiadone in humans was identified.

No experimental studies on the acute toxic effects of thiadone were identified.

4.2.2 Repeat dose toxicity

No information on the repeat dose toxicity of thiadone in humans was identified.

No experimental studies on the repeat dose toxicity of thiadone were identified.

Further searches were made for publicly available information on the toxicity of thiadone, including use of the online programme ChemIDPlus (US NLM, 2003). However, no information could be found.

Additional information was, therefore, sought through use of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1: Predicted toxicity data for thiadone using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$				
Flufenacet (experimental data)	598	3.74	LOEL: 1.2 mg/kg bw/day	Negative	Negative	Developmental effects in rats and rabbits
Thiadone (TOPKAT)	7.8 (1.2 – 51.1)	UE	MTD (feed/drink) UE MTD (oral gavage) UE	Negative	Positive	Negative
Thiadone (DEREK)	n/a	n/a	*Plausible* for skin sensitisation in humans	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software; UE-unreliable estimate

DEREK identified the presence of only one structural alert and the following conclusion was drawn:

It is considered plausible (i.e. there is a weight of evidence) that thiadone will exhibit skin sensitisation in some humans. This endpoint is predicted because thiadone is a hydrazine (or precursor) compound.

The analysis by TOPKAT suggested that thiadone was:

carcinogenic;
non-mutagenic; and
not a developmental toxicant

5 Guidelines and Standards

5.1 Flufenacet

The EC risk classification is: Xn-Harmful: R22, R48/22; Xi-Irritant: R43; N-Dangerous for the environment: R50, R53. Flufenacet has a WHO classification of 'slightly hazardous' and a US EPA classification of 'slightly toxic' (Lewis *et al.,* 2007).

An ADI of 0.004 mg/kg bw/day has been proposed (EC, 2003) using a safety factor (SF) of 300 (10x for interspecies extrapolation, 10x for intraspecies variability, and 3x for the lack of a NOAEL), based on a repeat dose toxicity/carcinogenicity dietary study in rats (US EPA, 1998).

5.2 Thiadone

Due to the lack of available human or experimental data, it has not been possible to establish a robust NOEL or NOAEL for thiadone. In addition, no ADI has been proposed for this metabolite by any authoritative organisation. Therefore, for the

purposes of this study a ‘project specific derived value’ (PSDV) has been derived for use in the risk assessment.

The predicted toxicity profile for FOE oxalate is limited, with only comparison of LD_{50} being possible; based on this the metabolite appears to have slightly higher activity to that of the parent. Therefore, – on a highly precautionary basis – it is proposed to adopt a PSDV of 0.004 mg/kg bw/day for thiadone (i.e. the same value as for the parent).

6 References

European Commission (EC, 2003) Review report for the active substance Flufenacet. Available at: http://ec.europa.eu/food/plant/protection/evaluation/newactive/list1_flufenacet_en.pdf

Lewis K, Green A, Tzilivakis J (2007) Pesticide database holding fate and ecotoxicological values. Report DL24 of the FP6 EU-funded FOOTPRINT project. Available at http://www.eu-footprint.org.

United States Environmental Protection Agency (US EPA, 1998); Pesticide Fact Sheet - Flufenacet. April, 1998. Available at: http://www.epa.gov/opprd001/factsheets/

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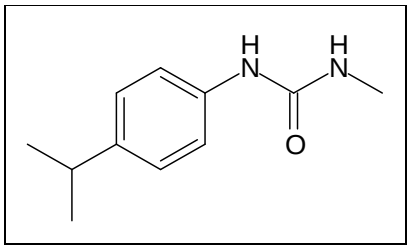
Appendix 12.15 Desmethylisoproturon (3-(4-isopropylphenyl)-1-methylurea

1 Introduction

Desmethylisoproturon is a major metabolite of the herbicide isoproturon (*N*,*N*-dimethyl-*N*'-[4-(1-methylethyl)phenyl]urea; CAS No. 34123-59-6).

The structure of desmethylisoproturon is presented in Figure 1.

Figure 1: Structure of desmethylisoproturon



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of isoproturon

Isoproturon is a selective pre- and post-emergence herbicide used to control annual grasses and annual broad leaved weeds in winter and spring wheat, winter and spring barley, winter rye and triticale. Isoproturon is sold worlswide under the trade names Alpha IPU 500; Alpha Isoproturon, Ingot, Koala, Trump, Javelin, Javelin Gold, Protugan and Tolugan Extra (PSD, 1995; Lewis *et al.,* 2007).

Isoproturon is often supplied as a soluble concentrate that is mixed with water and used as a spray (Lewis *et al.,* 2007).

2.2 Environmental fate

2.2.1 Parent

Isoproturon is moderately soluble in water (70.2 mg/L at 20^{o}C). In assessing the sorption to soil, a K_{oc} value of 139 L/ kg has been reported suggesting moderate mobility in the environment (Lewis *et al.,* 2007).

Degradation studies have shown isoproturon to be readily degraded in soils with a half-life of 12 and 23 days under laboratory (20^{o}C) and field conditions respectively. The compound is not considered to be persistent in the environment (Lewis *et al.,* 2007).

2.2.2 Metabolite: Desmethylisoproturon

Desmethylisoproturon is one of the major metabolites formed following hydrolysis of isoproturon, with an estimated maximum formation fraction of 0.140 (14%; Lewis *et al*., 2007).

Degradation studies have shown desmethylisoproturon to be degraded in soils with a half-life of 33 days under laboratory (20^{o}C) conditions. The metabolite is considered to be moderately persistent in the environment (Lewis *et al.,* 2007).

A K_{oc} value of 147 l/kg has been reported for desmethylisoproturon (Lewis *et al*., 2007) suggesting moderate mobility in the environment.

2.3 Potential routes of human exposure

Exposure of humans to the parent isoproturon may occur through ingestion of contaminated water, or during occupational handling. Due to isoproturon's low vapour pressure and short half-life in soil, it is unlikely that exposure via air is a major route of human exposure. Consumption of contaminated food is generally not considered a significant source of exposure to isoproturon for the general population. No measurable residues of isoproturon have been detected in grain samples using methods with detection limits of 0.1 to 0.01 mg/kg (WHO, 1996).

Exposure of humans to the metabolite desmethylisoproturon may occur through ingestion of the metabolite in water contaminated with isoproturon and its metabolites or form the metabolism of isoproturon following absorption of the parent compound.

Although no data on routes of exposure of humans to the metabolite desmethylisoproturon was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of isoproturon, or when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of isoproturon in humans.

Once absorbed isoproturon is widely and evenly distributed throughout the body with no accumulation (WHO, 1996).

In a single dose oral study, male and female rats of the RAI strain were given 5.52 or 5.69 mg/kg ^{14}[C]-isoproturon respectively; approximately 90% and 85% of the dose was excreted in urine in males and females, respectively, with 7 and 10% of dose respectively excreted in faeces. A half life of < 8 hr was determined (PSD, 1995). In male rats of the same strain, at a higher dose of 52.6 mg/kg of ^{14}[C]-isoproturon, the half life remained at about 8 hr and routes of excretion were similar (86 and 15% of dose in urine and faeces respectively).

Isoproturon was completely metabolised and largely excreted in the urine; the predominant metabolites are believed to have been formed by oxidation of the isopropyl group and N-demethylation. (PSD, 1995).

No information on the toxicokinetics of desmethylisoproturon was identified.

4 Toxicity Profile

4.1 Isoproturon

4.1.1 Acute Toxicity

No information is available on the acute toxicity of isoproturon in humans.

In rats, isoproturon has moderate acute toxicity; LD_{50}'s are 1826 mg/kg, 2000 mg/kg and 1.95 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis *et al.,* 2007).

Isoproturon is not an irritant to the eye or skin and is not considered a skin sensitizer (WHO, 1996).

4.1.2 Repeat dose toxicity

Principal organs of toxicity following repeated exposure of humans to isoproturon are the red blood cells and liver (EC, 2002).

Data on potential health effects following repeated exposure of humans to isoproturon has been reported on groups of workers involved in its manufacture. However, urine and blood analyses found no effect on peripheral blood count or evidence of haemolytic anaemia (WHO, 1996).

Experimentally, in rats fed diets containing isoproturon at up to 2000 mg/kg diet for two years, increased serum enzyme activities and cholesterol levels (indicative of hepatic enzyme induction) were noted at the highest dose. A marginal reduction in red blood cell parameters and increase in liver weight and incidence of hepatic acidophilic foci, at histopathology, were reported at the two highest doses. A NOAEL of 80 mg/kg (equivalent to 3.1 and 3.8 mg/kg bw/day) in males and females respectively, was established (WHO, 1996).

4.1.3 Carcinogenicity and mutagenicity

No information on the carcinogenic or mutagenic potential of isoproturon in humans was identified.

In Sprague-Dawley rats fed a diet containing isoproturon at up to 2000 ppm for two years, a decrease in food consumption and associated reduction in body weight gain were noted at the highest dose administered. At termination, higher liver weight was noted at 400 and 2000 ppm. Increased heptocholangiocarcinoma and cholangiocarcinoma were noted in rats of both sexes at 2000 ppm and in males at 400 ppm. Some treated females also developed hepatic haemangiosarcomas, a rare tumour, and this was considered to represent a treatment-related effect. At all doses investigated, males showed increased foci or areas of hepatic alteration; similar changes were also seen in females at 400 or 2000 ppm (PSD 1995). A NOAEL of 80 ppm (3.1 mg/kg bw/day) was established (EU, 2002).

Experimentally, isoproturon has been tested in *in vitro* and *in vivo* assays for mutagenicity. No evidence of mutagenicity has been demonstrated in bacterial, eukaryotic or *in vitro* and *in vivo* mammalian cells (WHO, 1996).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of isoproturon in humans was identified.

In a two-generation study in Wistar rats, isoproturon was administered in the diet at levels up to 400 ppm. F_0 males were treated for 10 weeks and females for 12 weeks prior to mating. Treatment was continuous until weaning of F_2 offspring. No effects were seen in F_0 animals. Reduced body weight gain was noted in F_1 parents and offspring at 400 ppm. Retarded spermatogenesis was seen in two F_1 males at 400 ppm and one F1 male at 200 ppm. Some males at 400 ppm also showed focal hyperplasia in the seminal vesicles and prostate. At the highest dose, a slight reduction in pregnancy rate was seen in F_1 animals. A reproductive NOAEL of 100 ppm (10 mg/kg/day) was established (PSD, 1995; WHO, 1996).

Teratology studies for isoproturon have been undertaken in female New Zealand White rabbits given isoproturon by oral gavage at up to 160 mg/kg/day from day 6 to day 18 of gestation. Maternal food consumption and body weight gain were reduced at the highest dose at which an increased number of runts (fetuses weighing < 70% of mean litter weight) was noted. Although the number of fetuses per dam was higher at the highest dose, this was associated with lower placental and fetal weights, and changes were not attributed to treatment. A developmental NOEL of 40 mg/kg/day was established (PSD, 1995, WHO, 1996).

4.2 Desmethylisoproturon

Searches were made for publicly available information on the toxicity of desmethylisoproturon, including use of the online programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK did not identify any structural alerts.

The analysis by TOPKAT suggested that desmethylisoproturon was:

carcinogenic;
non-mutagenic

Prediction of the reproductive and developmental toxicity of desmethylisoproturon using TOPKAT was not possible.

Table 1: Predicted toxicity data for desmethylisoproturon using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$ (range)				
Isoproturon (experimental data)	1826	1.95	NOAEL: 3.1 mg/kg/day Main target organs: Red blood cells and liver	Negative	Heptocholangiocar-cinoma, cholangioca-rcinoma and hepatic haemangiosarcoma (rats).	Evidence of retarded spermatogenesis and decreased pregnancy rate (rats). Increased number of runts (rabbits).
Desmethyliso-proturon (TOPKAT)	611 (142.6 - 2600)	345.9 (37.2 – 3200)	MTD (feed/drink) 5 mg/kg MTD (oral gavage) 5 mg/kg	Negative	Positive	UE
Desmethyliso-proturon (DEREK)	n/a	n/a	No alert	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software; UE- unreliable estimate

5 Guidelines and Standards

5.1 Isoproturon

The EC risk classification is: Carcinogen category 3: R40; N - Dangerous for the environment: R50, R53 and EU safety category is S2, S36/37, S60, S61. Isoproturon is classified by WHO as 'slightly hazardous' (Lewis *et al*., 2007).

An ADI of 0.015 mg/kg bw/day has been established (EU, 2002) for isoproturon using a safety factor (SF) of 200, based on a two year rat study in which Sprague-Dawley rats received a diet containing isoproturon at up to 2000 ppm Lewis *et al*., 2007). It should be noted however, that full study details were not available to fully assess the robustness of the SF applied in the study.

5.2 Desmethylisoproturon

Due to the lack of availability of experimental data, it has not been possible to establish a robust NOAEL for desmethylisoproturon, and no ADI has been published by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

Desmethylisoproturon is predicted by TOPKAT but not DEREK to retain the carcinogenic potential of the parent compound. The predicted acute oral toxicity and repeat dose oral MTDs (5 mg/kg bw/day) are also close to the respective values of the parent.

Given this, it is therefore proposed to adopt a PSDV of 0.015 mg/kg bw/day for desmethylisoproturon (i.e. the same value as for the parent). Based on the oral MTD predicted by TOPKAT, this will provide an overall SF of 333

6 References

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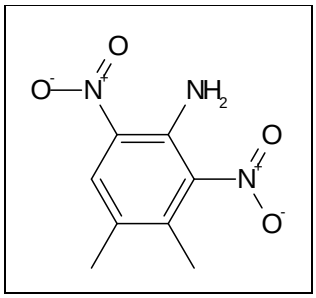
Appendix 12.16 2,6-Dinitro-3,4-xylidine

1 Introduction

2,6-Dinitro-3,4-xylidine is a major metabolite of the herbicide pendimethalin (*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine; CAS No. 40487-42-1).

The structure of 2,6-dinitro-3,4-xylidine is presented in Figure 1.

Figure 1: Structure of 2,6-dinitro-3,4-xylidine



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Pendimethalin

Pendimethalin is a selective herbicide used pre- and post-emergence to control annual grasses and annual broad-leaved weeds in field corn, potatoes, rice, cotton, soybeans, tobacco, peanuts and sunflowers (HSDB, 2003). Pendimethalin is sold worldwide under the trade names, Blazer, Bunker, Claymore, PDM 330 EC, PicoMax, Picona, PicoPro, Stomp, PicoStomp, Pendimethalin 330 EC and Trump (Lewis *et al.,* 2007).It is often supplied as an emulsifiable or emulsion concentrate that is mixed with water and used as a spray (Lewis *et al.,* 2007).

2.2 Environmental fate

2.2.1 Parent

Pendimethalin has low solubility in water (0.33 mg/L at 20^{o}C). In assessing the sorption to soil, a K_{oc} value of 15744 l/ kg has been reported suggesting that it is not mobile in the environment (Lewis *et al.,* 2007).

Studies have shown pendimethalin is moderately degraded in soils with half-lives of 123 and 90 days, under laboratory (20^{o}C) and field conditions respectively. The compound is moderately persistent in the environment (Lewis *et al.,* 2007).

During use, pendimethalin will be released into the atmosphere where it will be present in the vapour phase where it can react with photochemically-produced hydroxyl radicals; the estimated atmospheric half-life of 12.7 hr (HSDB, 2003).

2.2.2 Metabolite: 2,6-dinitro-3,4-xylidine

2,6-Dinitro-3,4-xylidine is one of the metabolites formed during degradation, and to a limit extent, metabolism of pendimethalin.

No information on the physicochemical properties or environmental fate of 2,6-dinitro-3,4-xylidine was identified.

2.3 Potential routes of human exposure

Workers may be exposed to pendimethalin primarily through dermal contact, inhalation or ingestion of aerosols during mixing and application. Workers and

bystanders may also be subject to dermal contact with treated plants and soil. The general population is most likely to be exposed to pendimethalin through dermal contact or ingestion of contaminated water (including rain water); this is most likely near agricultural areas during the growing season (HSDB, 2003).

Although no data on routes of exposure of humans to the metabolite 2,6-dinitro-3,4-xylidine was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of pendimethalin, or to a much lesser extent, when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of pendimethalin in humans.

In a single dose oral study in rats given 7.3 or 37 mg/kg ^{14}[C]-pendimethalin, absorption from the GI tract was very low and a large proportion was excreted unchanged in the faeces. Once absorbed, pendimethalin was distributed widely in the body but at a minimal level; concentrations were 0.3 ppm in all tissues examined except for fat where a level of 0.9 ppm was noted four days after administration (Zulalian, 1990). Of the absorbed pendimethalin, rapid metabolism occurred in the kidneys and liver with excretion via the urine; metabolism involved hydroxylation of the 4-methyl and the N-1-ethyl group followed by oxidation of the alkyl groups to carboxylic acids, nitro reduction, cyclization and subsequent conjugation (Zulalian, 1990). By 24-hours after the 37 mg/kg dose, over 90% of the administered dose had been eliminated via the faeces or urine; recovery rose to 96% by four days (Zulalian, 1990).

No information is available on the toxicokinetics of 2,6-dinitro-3,4-xylidine in humans.

4 Toxicity Profile

4.1 Pendimethalin

4.1.1 Acute Toxicity

No information is available on the acute toxicity of pendimethalin in humans.

In rats, pendimethalin has moderate acute toxicity; LD_{50}'s are 3189 mg/kg, 2000 mg/kg and 320 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis *et al.,* 2007).

Pendimethalin is not a skin irritant or sensitizer in rabbits or guinea pigs, but causes mild eye irritation in rabbits. Inhalation of dusts or fumes may be mildly to moderately irritating to the linings of the mouth, nose, throat, and lungs (EXTOXNET, 1996).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of pendimethalin in humans was identified.

Experimentally, the principal target organs of repeated exposure for pendimethalin are the liver and, in rats only, the thyroid (EC, 2003).

In Beagle dogs fed for two years on diets containing pendimethalin at up to 200 mg/kg bw/day, serum alkaline phosphatase (SAP) activity was increased at the two highest doses. Increased liver weight and associated inflammatory changes and haemosiderosis were also noted in the two highest dose groups. A NOEL of 12.5 mg/kg/day was established, based on the hepatic toxicity (IRIS, 1991).

In rats fed pendimethalin in the diet for 56 days for study of thyroid function only, exposure to 500 ppm (31 mg/kg bw/day) caused changes in serum chemistry by day

28 ; these comprised statistically significant decreases (38%) in total serum T4, reverse T3 (25%) and total T4 (28%) and an increase in free T3 (13%).

4.1.3 Carcinogenicity and mutagenicity
No information on the carcinogenic and mutagenic potential of pendimethalin in humans was identified.

In the 56 day rat thyroid function study reported above, histopathological changes included increased thyroid follicular cell height (40%) and decreased colloid areas (37%); some of these changes were detectable by day 3 of treatment. Although such changes may be indicative of carcinogenic potential, no tumour diagnosis was apparent in the study. However, a NOEL of 100 ppm (10 mg/kg/day) was established for potential carcinogenic effects (CEPA, 1999) and pendimethalin has been classified by the US EPA as a Group C, *possible human carcinogen* (US EPA, 1997).

In mice fed a diet containing 75 mg/kg bw/day of pendimethalin for 18 months, no evidence of increased tumour formation was reported (EXTOXNET, 1996).

Experimentally, pendimethalin was negative in a number of *in vitro* and *in vivo* mutagenicity assays including tests on live animals and mammalian and bacterial cell cultures (EXTOXNET, 1996).

4.1.4 Reproductive and development toxicity
No information was identified on the reproductive or developmental effects of pendimethalin on humans.

Experimentally, in a three-generation rat study in which pendimethalin was administered via the diet at up to 250 mg/kg/day, reduced offspring numbers at birth and offspring growth (from weaning to maturity) were noted at the mid and high doses. No effects were observed below 30 mg/kg/day, and a reproductive NOEL of 25 mg/kg/day was established (IRIS, 1991).

Teratology studies in pregnant rats administered pendimethalin in the diet at up to 500 mg/kg bw/day have been reported. No fetotoxic or teratogenic effects were noted and a NOAEL of 500 mg/kg bw/day was established (IRIS, 1991).

4.2 2,6-dinitro-3,4-xylidine
No information on the repeat dose toxicity of 2,6-dinitro-3,4-xylidine in humans was identified.

Searches were made for publicly available information on the toxicity of 2,6-dinitro-3,4-xylidine, and by use of the online programme ChemIDPlus (US NLM, 2003). However, the information obtained was extremely limited.

Additional information was, therefore, sought through use of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1: Predicted toxicity data for 2,6-dinitro-3,4-xylidine using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Pendimethalin (experimental data)	3189	320	NOAEL: 12.5 mg/kg/day Main target organs: Liver and, in rats, thyroid	Negative	Some evidence in rats (thyroid).	Negative
2,6-dinitro-3,4-xylidine (TOPKAT)	355.8 (87.7-1400)	2800 (366.1-10,000)	MTD (feed/drink) 58.3 mg/kg MTD (oral gavage) 58.3 mg/kg	UE	Positive	Negative
2,6-dinitro-3,4-xylidine (DEREK)	n/a	n/a	*Plausible* for hepatotoxicity; methaemoglob-inaemia; skin sensitisation in humans	*OPEN* in humans	*Plausible* in humans	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software; UE- unreliable estimate

DEREK identified the presence of a number of structural alerts in the molecule, and the following conclusions were drawn:

It is considered plausible (i.e. there is a weight of evidence) that 2,6-dinitro-3,4-xylidine will exhibit carcinogenicity in humans. This endpoint is predicted because 2,6-dinitro-3,4-xylidine is an aromatic nitro compound;
It is considered plausible (i.e. there is a weight of evidence) that 2,6-dinitro-3,4-xylidine will exhibit hepatotoxicty in humans. This endpoint is predicted because 2,6-dinitro-3,4-xylidine is an aromatic nitro compound;
It is considered plausible (i.e. there is a weight of evidence) that 2,6-dinitro-3,4-xylidine will exhibit methaemoglobinaemia in humans. This endpoint is predicted because 2,6-dinitro-3,4-xylidine is a simple aniline or precursor;
It is considered OPEN (i.e. there is no weight of evidence for or against) that 2,6-dinitro-3,4-xylidine will exhibit mutagenicity in humans. This endpoint is predicted because 2,6-dinitro-3,4-xylidine is a aromatic nitro compound; and
It is considered plausible (i.e. there is a weight of evidence) that 2,6-dinitro-3,4-xylidine will exhibit skin sensitisation in humans. This endpoint is predicted because 2,6-dinitro-3,4-xylidine is an aromatic primary or secondary amine.

The analysis by TOPKAT also suggested that 2,6-dinitro-3,4-xylidine was:

carcinogenic
not a developmental toxicant.

Prediction of the mutagenic potential of 2,6-dinitro-3,4-xylidine using TOPKAT was not possible.

5 Guidelines and Standards

5.1 Pendimethalin

The EC risk classification is: Xi - Irritant: R43; N - Dangerous for the environment: R50, R53 and EU safety category is S2, S22, S29, S37, S60, S61. Pendimethalin is classified by WHO as 'slightly hazardous' and the US EPA as 'slightly toxic' (Lewis *et al.,* 2007).

An ADI of 0.125 mg/kg bw/day was established for pendimethalin (EU, 2003) using a safety factor (SF) of 100, based on the two feeding study in Beagle dogs administered pendimethalin at up to 200 mg/kg bw/day (Lewis *et al*., 2007).

5.2 2,6-dinitro-3,4-xylidine

Due to the lack of availability of experimental data, it has not been possible to establish a robust NOEL or NOAEL for 2,6-dinitro-3,4-xylidine, and no ADI has been published by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The toxicity profile for 2,6-dinitro-3,4-xylidine is overall predicted to be similar to that of the parent. The highest oral MTD predicted by TOPKAT is 58.3 mg/kg bw/day (irrespective of method of oral administration). Applying a SF of 100 would give a nominal value of 0.58 mg/kg bw/day, which would be higher than the established ADI of 0.125 mg/kg bw/day for the parent pendimethalin. Given the predicted hepatotoxicity and concerns regarding potential mutagenic and carcinogenic potential raised by the predictive systems, it is proposed to adopt a PSDV of 0.125 mg/kg bw/day for 2, 6-dinitro-3,4-xylidine (i.e. the same value as for the parent); this will provide an overall SF of 466.

6 References

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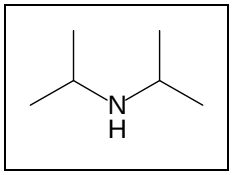
Appendix 12.17 Diisopropylamine

1 Introduction

Diisopropylamine (CAS No. 108-18-9) is used as a chemical intermediate and catalyst. It is also a major metabolite of the herbicide tri-allate *S*-(2,3,3-trichloro-2-propenyl)bis(1-methylethyl)carbamothioate; CAS No. 2303-17-5).

The structure of diisopropylamine is presented in Figure 1.

Figure 1: Structure of diisopropylamine



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of tri-allate

Tri-allate is a soil-acting herbicide used pre- and post-planting for control of wild oats in barley, lentils, peas and spring and winter wheat (IPCS, 1994). It is sold worldwide under the trade names Avadex Excel, Buckle, Carbamothic acid, CP 23426, Dipthal, Far-Go, Showdown and TDTC Technical, and is supplied as granules for incorporation into the soil or direct surface application (EXTOXNET, 1996; Lewis *et al.,* 2007).

2.2 Use of Diisopropylamine

Diisopropylamine is a chemical intermediate and catalyst used in the synthesis of pesticides and pharmaceuticals.

2.2 Environmental fate

2.2.1 Parent

Tri-allate has low solubility in water (4.1 mg/L at 20^{o}C). In assessing the sorption to soil a K_{oc} value of 4301 L/ kg has been reported (Lewis *et al.,* 2007) suggesting that it is not mobile in the environment.

Studies have shown tri-allate to be moderately degraded in soils, with half-lives of 58.3 and 46 days under laboratory (20^{o}C) and field conditions respectively. The compound is considered to be moderately persistent in the environment (Lewis *et al.,* 2007).

Use of tri-allate will result in its release into the environment. If released to air, tri-allate will occur solely in the vapor-phase in the ambient atmosphere where it will be degraded by reaction with photochemically-produced hydroxyl radicals; the half-life for this reaction is estimated to be 12 hrs. (HSDB, 2003).

2.2.2 Metabolite: Diisopropylamine

Diisopropylamine has low solubility in water (1.1 x10^{5} mg/L at 25^{o}C). In assessing the sorption to soil, a K_{oc} value of 140 l/ kg has been estimated suggesting that it has high mobility in the environment (HSDB, 2009).

Production and use of diisopropylamine may result in its release into the environment. If released to air, diisopropylamine will exist solely in the vapor-phase in the ambient atmosphere where it will be degraded by reaction with photochemically-

produced hydroxyl radicals; the half-life for this reaction is estimated to be 12 hrs. (HSDB, 2009).

2.3 Potential routes of human exposure

Workers may be exposed to tri-allate primarily through dermal contact, inhalation or ingestion of aerosols during mixing and application. Workers and bystanders may also be subject to dermal contact with treated plants and soil (IPCS, 1994).

Although no data on routes of exposure of humans to the metabolite diisopropylamine was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of tri-allate, or to a much lesser extent, when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics

3.1 Tri-allate

No information is available on the toxicokinetics of tri-allate in humans.

In a single dose oral study in male and female Sprague-Dawley rats dosed with radiolabeled tri-allate at 5 or 500 mg/kg bw, tri-allate was readily absorbed; 92% and 96% of administered dose was recovered for the low and high dose respectively by 72 hr. In the blood, absorbed tri-allate was principally associated with haemoglobin; its distribution was consistent with a two compartment open model and the half-life of the initial phase was 5.9 - 22.8 hr while the terminal phase was 171 - 265 hr. Metabolism of tri-allate comprised three main pathways:

S-oxidation leading to sulphur acids,
S-oxidation/hydrolysis/reduction leading to thiol derivatives, and
C-oxidation of the 2,3,3-trichloropropenethiol moiety.

The thiol served as a precursor of metabolites with an unoxidized sulfur atom; sulfur acids formation was the principal metabolic route at 5 mg/kg while thiol production and carbon oxidation became more significant at 500 mg/kg level. This finding is consistent with a saturation of the S-oxidation pathway. Unchanged tri-allate was also excreted at the high (500 mg/kg) dose. Overall, approximately 42 - 52% of the dose was excreted in the urine, 33 - 46% in the faeces and approximately 4% of the dose in expired air. The authors suggest that elevated excretion of tri-allate and C-oxidation metabolites should be considered when interpreting findings of high dose tri-allate feeding studies because mutagenic effects have been associated with products of C-oxidation pathways (Ridley et al., 1993; Nadeau et al., 1993).

3.2 Diisopropylamine

No information on the toxicokinetics of diisopropylamine in humans was identified.

4 Toxicity Profile

4.1 Tri-allate

4.1.1 Acute toxicity

No information on the repeat dose toxicity of tri-allate in humans was identified.

In rats, tri-allate has low acute toxicity; LD_{50}'s are 3455 mg/kg, 5000 mg/kg and 5.3 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis *et al.,* 2007).

A study in rats fed diet containing tri-allate at 50 to 2000 mg/kg bw/day has identified a range of effects associated with acute tri-allate exposure. These included abnormal behaviour at doses of ≥ 100 mg/kg and reduced body weight and deaths at ≥ 600 mg/kg. In sheep, symptoms of tri-allate poisoning including depression, lack of appetite, watering mouth, weakness and convulsions were seen at doses ≥ 300 mg/kg bw (EXTOXNET, 1996).

Although tri-allate is a carbamate, it does not inhibit cholinesterase activity in rats administered a single oral dose (route unknown) even at high dosages of 1500 - 3000 mg/kg bw (EXTOXNET, 1996).

Tri-allate is moderately irritating to the eye and is a mild skin irritant. Tests on guinea pigs indicate that triallate does not cause allergic skin reactions (EXTOXNET, 1996).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of tri-allate in humans was identified.

Experimentally, the principal target organs of repeated exposure in rats, mice, hamsters, rabbits and dogs are the liver and kidneys (IPCS, 1994).

In Beagle dogs fed a diet containing tri-allate at levels designed to achieve doses of 0, 1.5, 5 or 15 mg/kg diet/day (ad libtum) for two years (overall achieved intakes in treated groups were 85% of the target dose, i.e. 1.275, 4.25 and 12.75 mg/kg bw/day), increased liver weights were seen in males at the highest dose and in females given the mid- and high dose. Increases in hemosiderin deposits, particularly in the spleen, were observed in all treated groups while an increase in serum alkaline phosphatase activity was noted at mid-and high-doses. A NOAEL of 1.275 mg/kg day was established in this study (IPCS, 1994).

In a two year feeding study in mice at 3 and 12.5 mg/kg bw/day, increased liver and heart weights, histopathological changes in the liver and spleen and mineralisation in the brain and cornea were noted; a NOEL for tri-allate of 3 mg/kg bw/day was reported (IRIS, 1992).

Hamsters fed tri-allate in the diet at up to 100 mg/kg bw/day for 22 months showed decreased body weight gain, altered blood chemistry, slight anaemia, and increased liver and decreased spleen weights. A NOEL of 5 mg/kg bw/day was reported (EXTOXNET, 1996)

Chronic exposure to tri-allate in rats has been shown to result in two distinct types of neurological effects, (i) central nervous system effects with associated histopathological changes that are reversible at lower exposure levels, (ii) neurotoxicity associated with myelin loss in the spinal cord and some peripheral nerves resulting in decreased grip strength and increased landing foot splay (IPCS, 1994).

4.1.3 Carcinogenicity and mutagenicity

No information on the carcinogenic or mutagenic potential of tri-allate in humans was identified.

In chronic oral dosing studies, tri-allate did not increase the incidence of tumours in rats fed treated diet containing up to 12.5 mg/kg bw/day for two years and in hamsters fed 100 mg/kg bw/day for 22 months (EXTOXNET, 1996).

Tri-allate has been tested in a number of *in vivo* and *in vitro* assays. *In vitro*, in bacterial and animal cells, positive and negative result have been found suggested that tri-allate is either non-mutagenic or only weakly mutagenic in vitro. However, in *in vivo* tests on fruit flies, hamsters and mice, findings were negative (EXTOXNET, 1996).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of tri-allate in humans was identified.

In a two-generation study in rats administered tri-allate in the diet at 30 mg/kg bw/day over the mating, pregnancy and lactation periods, reproductive effects were noted. These included reductions in maternal body and pup weight, pregnancy rate and duration and level of pup survival. Tri-allate is therefore a reproductive toxicant, at least at high doses (ETOXNET, 1996).

Teratology studies have been carried out in pregnant rats fed diets containing tri-allate at up to 90 mg/kg bw/day on days 6 to 20 of gestation. Despite evidence of toxic effects in dams and offsping given the high dose, no teratogenic changes were seen (EXTOXNET, 1996).

4.2 Diisopropylamine

4.2.1 Acute Toxicity

In humans experiencing occupationally-exposures to high atmospheric levels of diisopropylamine vapour, acute effects may included: transient dimness of vision; nausea; and headaches. Visual disturbance occurs within two to three hours of exposure and may persist for one to two hours after removal from exposed conditions (HCN, 2003).

In rats, diisopropylamine has moderate to low acute toxicity; LD_{50}'s are 770 mg/kg, 500 mg/kg and 4800 mg/kg, by the oral, dermal or inhalation routes respectively (HSDB, 2009).

Diisopropylamine is irritating to the eye and may cause disturbance of vision at 25 and 50 ppm (HSDB, 2009).

4.2.2 Repeat Dose Toxicity

No information on the effects of repeated exposure to diisopropylamine in humans was identified.

In Sprague-Dawley rats exposed to diisopropylamine vapour at up to 2000 mg/m^3 for six hours/day, five days/week, for one month, one male and two females in the high dose group died. Signs of toxicity in treated rats included respiratory distress, mucous membrane irritation and non-responsiveness at the high-dose and a decrease in body weight, compared to controls, at the mid- and high-doses. Other changes included: increased eythrocyte count and haemoglobin and haematocrit values in males at the high dose and females at mid and high doses, and all treated males had significantly reduced leukocyte counts (28 - 33%) attributable to reductions in lymphocyte numbers. Although intergroup differences in blood albumin, total protein, and cholesterol levels and altered alkaline phosphatase and/or serum glutamic pyruvic transaminase activities, were seen, these were not considered to be treatment related. At necropsy, animals exposed to 2000 mg/m^3 showed changes in organ weights (including increased relative adrenal gland, heart, and kidney weights

and decreased relative spleen weights in both sexes and increased relative liver weights in females). In all rats exposed to 600 or 1000 mg/m^3 and most exposed to 100 mg/m^3, hyperplasia and metaplasia of the nasal turbinates was noted. Inflammation, mucosal erosion/ulceration and necrosis/dissolution of turbinate septal cartilage or bone were also observed at 1000 mg/m^3 and in most animals at 600 mg/m^3. Almost all rats exposed to 2000 mg/m^3 had lesions of the trachea (mucosal epithelial hyperplasia/metaplasia and inflammation) and lungs (bronchiolar epithelial hyperplasia/metaplasia). A NOAEL could not be established due the effects seen at 100 mg/m^3 (HCN, 2003).

4.2.3 Carcinogenicity and mutagenicity

No information on the carcinogenic or mutagenic potential of diisopropylamine in humans was identified.

Evidence of metaplasia apparent in Sprague-Dawley rats exposed to diisopropylamine vapour at up to 2000 mg/m^3 for six hours/day, five days/week, for one month, may be attributed to its high irritancy, rather than representing a direct genotoxic effect (HCN, 2003).

A *Salmonella typhimurium* study (5 strains) with or without metabolic activation at doses of up 10 mg/plate was negative (HSDB, 2009). Diisopropylamine was also negative in a DNA-repair assay in cultured rat hepatocytes at levels of 0.1 - 5000 µg/mL (preliminary assay) and 10 - 2500 µg/mL (replicated main assay); it was however cytotoxic at 5000 µg/mL. (HCN, 2003).

4.2.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of diisopropylamine in humans was identified.

Additional information was sought through use of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Summaries of the toxicity data predicted for Diisopropylamine from these tools are summarised below (Table 1).

Table 1 Predicted toxicity data for diisoppropylamine using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity/ Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Tri-allate (experimental data)	3455	5.3	NOAEL 1.275 mg/kg/d Main target organs: Liver and kidney	Negative or weakly mutagenic (*in vitro*)	Negative	Reduced body and pup weights, reduced pregnancy rate and length, and reduced pup survival.
Diisopropyl-amine (experimental data)	770	4800	LOAEL 100 mg/m^3. Main target organ: respiratory system	Negative	Metaplasia apparent following inhalation (rats)	NR
Diisopropyl-amine (TOPKAT)	531.4 (103.6-2700)	10,000 (671.6-10,000)	MTD (feed/drink): UE MTD (oral gavage): UE	Negative	Positive	Positive
Diisopropyl-amine (DEREK)	n/a	n/a	No alert	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software; UE- unreliable estimate; NR- not reported

DEREK did not identify the presence of any structural alerts.

The analysis by TOPKAT suggested that diiospropylamine was:

carcinogenic;
non-mutagenic; but
a developmental toxicant.

5 Guidelines and Standards

5.1 Tri-allate

The EC risk classification is: Xn – harmful: R22, R48/22; Xi - Irritant: R43; N - Dangerous for the environment: R50, R53 and EU safety category is S2, S24, S37, S60, S61. Tri-allate is also classified by WHO as 'slightly hazardous' and the US EPA as 'slightly toxic' (Lewis *et al.,* 2007).

An ADI of 0.025 mg/kg bw/day has been established for tri-allate (EFSA, 2008; EU, 2009; Lewis *et al*., 2007) using a safety factor (SF) of 100, based on a two year rat feeding study (full study details not available).

5.2 Diisopropylamine

It has not been possible to establish a robust NOAEL for diisopropylamine, and no ADI has been published by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

A LOAEL of 100 mg/m^3 was noted in a one month inhalation study in rats. However, this was based on a number of changes in the respiratory system that were probably attributable to local irritancy. At this exposure level, systemic effects were not however observed. In addition, the main toxic effects of diisopropylamine result via inhalation exposure; exposure through drinking water is likely to have minimal toxic effects. Both the available experimental data and the TOPKAT predictions suggest that the metabolite may have a greater acute toxic potential than the parent tri-allate, and TOPKAT has also raised concerns with regard to the carcinogenic potential of diisopropylamine. However, no estimate of repeat dose oral toxicity is available for diisopropylamine. Given this, using route to route extrapolation (IGHRC, 2006) and based on a LOAEL of 100 mg/m^3, it may be estimated that the equivalent oral dose might be 100 mg/kg bw/day. Applying a SF of 1000 would give a PSDV of 0.1 mg/kg bw/day which is higher than the ADI of 0.025 mg/kg bw/day established for the parent tri-allate.

Therefore, – on a highly precautionary basis – it is proposed to adopt a PSDV of 0.025 mg/kg bw/day for diisopropylamine (i.e. the same value as for the parent). This will give an overall SF of 4000.

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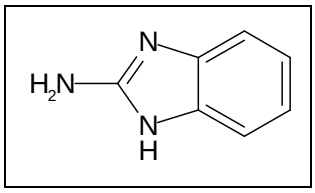
Appendix 12.18 2-Aminobenzimidazole

1 Introduction

2-Aminobenzimidazole (CAS No. 934-32-7) is a major metabolite of the fungicide carbendazim (methyl 1-H-benzimidazol-2-yl carbamate; CAS No. 10605-21-7) that is formed during plant metabolism and in soil (HSDB, 2003); the metabolite is also formed during metabolism of absorbed carbendazim in humans.

The structure of 2-aminobenzimidazole is presented in Figure 1.

Figure 1: Structure of 2-aminobenzimidazole



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Carbendazim

Carbendazim is a broad spectrum benzimidazole carbamate systemic fungicide that is sold worldwide under the trade names Delsene 50 Flo, Harvesan and Mascot. The fungicide is sold as a colourless crystalline powder that is mixed with water and applied as a spray or used as a drench or pre-planting dip (Lewis *et al*., 2007). It is used in the control of a wide range of pathogens on cereals, fruits, ornamentals and vegetables and post-harvest during food storage (PSD, 1992).

2.2 Environmental fate

2.2.1 Parent

Carbendazim has low water solubility (8 mg/L at 20°C; Lewis *et al*., 2007). In assessing the sorption to soil carbendazim has a K_{oc} value of 223L/kg^{-1} suggesting it is moderately mobile in the environment (Lewis *et al*., 2007).

Studies have shown carbendazim to degrade in soils, with a half-life of 18 days under field conditions. The fungicide is considered to be non-persistent in the environment (Lewis *et al*., 2007).

Carbendazim's production may result in its release into waste streams while its use as a fungicide will result in direct release into the environment. If released to air, carbendazim will exist solely in the particulate phase in the atmosphere with this particulate-phase carbendazim being lost from the atmosphere by deposition (HSDB, 2003).

2.2.2 Metabolite: 2-aminobenzimidazole

2-aminobenzimidazole is formed during the degradation and metabolism of carbendazim; the estimated maximum formation fraction is 0.08 (8%; Lewis *et al*., 2007).
A K_{oc} value of 14,900 Lkg^{-1} has been reported for 2-aminobenzimidazole (Lewis *et al*., 2007) suggesting high mobility in the environment.

No further information on the physiochemical properties or environmental fate of 2-aminobenzimidazole was identified.

2.3 Potential routes of human exposure

Exposure of humans to the parent compound carbendazim may occur through ingestion of contaminated food and water or through inhalation or dermal contact during occupational handling (HSDB, 2003).

Although no data on routes of exposure of humans to the metabolite 2-aminbenzimidazole was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of carbendazin, when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics
No information is available on the toxicokinetics of carbendazim in humans.

In rats administered a single dose of 14[C]-carbendazim at 12 mg/kg bw, absorption from the GI tract was found to be approximately 80 - 85% (Krechniak, 1986).

The metabolic fate of 14[C]-carbendazim was assessed following i.v. administration. Two metabolites 2-aminbenzimidazole and methyl 5-hydroxy-2-benzimidazolecarbamate (5-HBC) were rapidly formed, with peak concentrations occurring in the liver and kidneys by 15 min after administration. Unchanged carbendazim was present at the highest concentration in blood; 5-HBC accumulated in tissues while 2-aminbenzimidazole was found in only minor amounts (Krechniak, 1986). The radiolabel was predominantly excreted in the urine (65%) with 35% excreted in faeces (Krechniak, 1986).

4 Toxicity Profile
4.1 Carbendazim
4.1.1 Acute Toxicity
Acute dermal exposure of humans to carbendazim has been reported to cause moderate toxicity whilst ingestion causes only mild toxic effects (HSDB, 2003).

Carbendazim has low acute toxicity in rats with LD_{50}'s of 5000 mg/kg, 2000 mg/kg and 5.6 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis *et al*., 2007).

Carbendazim is not an irritant to the eye or skin, and is not a skin sensitizer (PSD, 1992).

4.1.2 Repeat dose toxicity
No information on the repeat dose toxicity of carbendazim in humans was identified.

In Wistar rats fed diets containing carbendazim at up to 5000 ppm (equivalent to approximately 250 mg/kg bw/day) for two years, no treatment-related effects were noted in food consumption or survival. At the highest dose, bodyweight was slightly reduced in females from week 12 as was haemoglobin content from week 26. Other effects in females receiving the top dose of carbendazim included decreased packed cell volume and total serum protein level and increased serum alkaline phosphatase (ALP) and alanine transaminase (ALT) activities. In males, blood urea nitrogen levels were elevated at the highest dose, as were the liver weights of both sexes. A NOEL of 300 ppm (15 mg/kg bw/day) was established (PSD, 1992).

In CD-1 mice administered carbendazim in the diet at up to 7500 mg/kg bw/day for 73 weeks, showed increased deaths, hepatic centrilobular hypertrophy, necrosis and swelling, thymic lymphoid depletion and reduced weight, and reduced kidney weight associated with the deposition of a yellow-brown pigment in the tubules at 1500

mg/kg/day. At the highest dose (7500 mg/kg/day) food consumption was increased but food utilisation efficiency was impaired, and erythrocyte count and blood haemoglobin concentration were reduced. Accumulation of pigment in the renal tubules together with the presence of macrophages and cystic tubules were also noted (PSD, 1992).

4.1.3 Carcinogenicity and mutagenicity

No information on the carcinogenic and mutagenic potential of carbendazim in humans was identified.

Following histopatholgical examination female Wistar rats administered carbendazim in the diet for two years (see above) showed a possible treatment-related increase in diffuse proliferation of parafollicular cells of the thyroid at the top dose used (250 mg/kg bw/day). No other incidence of neoplastic lesions was seen (PSD, 1992).

In a 73 week feeding study in CD-1 mice (see above), carbendazim treatment resulted in an increased incidence of benign and malignant hepatic tumours; a dose-related decrease in latency period of tumour formation was noted. In females, these hepatic changes were apparent at doses of 500 mg/kg/day or above while, in males, such changes were only apparent at 1500 mg/kg/day or above (PSD, 1992).

Several *in vitro* and *in vivo* tests suggest that carbendazim is not mutagenic although it may act as an *in vitro* mitotic spindle poison in mammalian cells. However, no such adverse effect has been reported *in vivo* (IPCS, 1996).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of carbendazim in humans was identified.

In a three-generation reproductive study, CD rats were given carbendazim in the diet at up to 10,000 ppm. A reduced average litter weight at weaning was noted in all generations at 5000 and 10,000 ppm. However, there was no other evidence of embryo-and fetotoxicity. The NOEL was estimated to be 500 ppm (approximately 25 mg/kg bw/day; IPCS, 1996).

In a study of male reproductive toxicity in Sprague-Dawley rats, significant testicular and efferent duct changes were noted two days after a single oral dose of 100 mg/kg bw. The testicular atrophy observed at 70 days was attributed to occlusion of the efferent ductules. A NOAEL of 50 mg/kg bw was established (IPCS, 1996).

Studies of developmental toxicity have been undertaken in rats and rabbits. In three studies in rats, treatment by oral gavage resulted in maternal toxicity (clinical signs, decreased body weight gain and abortion) at 60 mg/kg bw/day or above. Developmental effects were also seen at 20 mg/kg bw/day or above; these comprised decreased foetal weight and increased percentage of fetuses with variations per litter. The latter effects were attributed to a delay in development since they correlated with the reduction in fetal weight. Malformations identified included hydrocephaly, anophthalmia, microphthalmia, axial skeletal malformations and malformed scapulae, which were significantly increased at 30 mg/kg bw/day or above in two studies and at 90 mg/kg bw/day or above in the other study. A slightly higher incidence of skeletal malformations were also noted at 20 mg/kg bw/day. A NOEAL for maternal toxicity of 30 mg/kg bw/day and a developmental NOAEL of 10 mg/kg bw/day were established, with the threshold for embryo/fetotoxicity and teratogenicity considered to be 20 mg/kg bw/ day (IPCS, 1996).

In a developmental toxicity study in rabbits subject to oral gavage, maternal toxicity (reduced food consumption and body weight gain and abortion) was apparent at the highest dose tested (125 mg/kg bw/day). A decrease in implantation rates and size of live litters and an increase in resorptions were noted at 20 and 125 mg/kg bw/day. Other changes noted comprised decreased fetal body weight and increased incidence of malformations (cervical vertebrae, ribs and thoracic vertebrae) at 125 mg/kg bw/ day. NOAELs of 20 and 10 mg/kg bw/day were established for maternal and developmental toxicity respectively (IPCS, 1996).

4.2 2-Aminbenzimidazole

No information on the repeat dose toxicity of 2-aminbenzimidazole in humans was identified.

Searches were made for publicly available information on the toxicity of 2-aminbenzimidazole, including use of the online programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1: Predicted toxicity data for 2-aminbenzimidazole acid using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Carbendazim (experimental data)	5000	5.6	NOEL 15.0 mg/kg/d Main target organs: Liver and Thyroid	Genotoxic potential not established	Liver carcinogen (rat; mice)	Decreased spermatogen-esis. Decreased foetal weight and increase in malformations.
2-aminbenzimi-dazole (TOPKAT)	UE	5300 (681.5-10,000)	MTD (feed/drink) 1800 mg/kg MTD (oral gavage) 1800 mg/kg	Negative	Negative	Indeterminate
2-aminbenzimi-dazole (DEREK)	n/a	n/a	No alert	*Open* in humans	*Plausible* in humans	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software; UE-unreliable estimate

DEREK identified the presence of a number of structural alerts and the following conclusions were drawn:

It is considered plausible (i.e. there is a weight of evidence) that 2-aminbenzimidazole will exhibit carcinogenicity in humans. This endpoint is predicted because 2-aminbenzimidazole is an aromatic amine or amide.

It is considered open (i.e. there is uncertainty in the evidence) that 2-aminbenzimidazole will exhibit mutagenicity in humans. This endpoint is predicted because 2-aminbenzimidazole is an aromatic amine or amide and a benzimidazole.

The analysis by TOPKAT indicated that 2-aminbenzimidazole was:

non-carcinogenic; and
non-mutagenic.

Prediction of the reproductive and developmental toxicity of 2-aminbenzimidazole using TOPKAT was not possible.

5 Guidelines and Standards

5.1 Carbendazim

The EC risk classification is: Mutagenic category 2: R46; Reproduction risk category 2: R60, R61; N – Dangerous for the environment: R50, R53. The EC safety categories are S45, S53, S60 an S61. Carbendazim is also classified by WHO as 'unlikely to present acute hazard in normal use' and by the US EPA as slightly toxic' (Lewis *et al.,* 2007).

An ADI of 0.02 mg/kg bw/day has been established for carbendazim (EFSA, 2008) using a safety factor of 500, based on a developmental NOAEL of 10 mg/kg bw/day established for rats and rabbits.

5.2 2-aminobenzimidazole

Due to the lack of experimental data, it has not been possible to establish a robust NOEL or NOAEL, for 2-aminobenzimidazole, and an ADI has not been published by an authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

Overall, the toxicity profile for 2-aminobenzimidazole appears, on the basis of the predictive software to be somewhat less than that of the parent. The highest oral MTD predicted by TOPKAT for 2-aminobenzimidazole is 1800 mg/kg bw/day (irrespective of type of oral administration). Applying a SF of 100 would give a nominal value of 18 mg/kg bw/day. This is significantly above the established ADI of 0.02 mg/kg bw/day for the parent carbendazim. Given this, it is – on a highly precautionary basis – therefore proposed to adopt a PSDV of 0.02 mg/kg bw/d for 2-aminobenzimidazole (i.e. the same value as for the parent); this will provide an overall SF of 900. However, it should be noted that the ADI for carbendazim will reflect concerns regarding the carcinogenic potential and reproductive and developmental toxicity.

6 References

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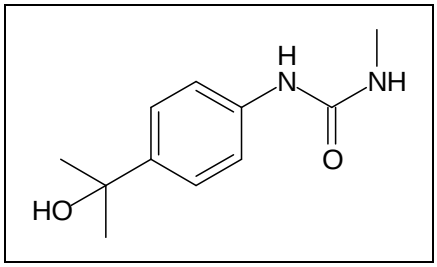
Appendix 12.19 3-[4-(2'-Hydroxy-2'- propyl)-phenyl]-methyl urea

1 Introduction

3-[4-(2'-hydroxy-2'- propyl)-phenyl]-methyl urea is a metabolite of the herbicide isoproturon (*N*,*N*-dimethyl-*N*'-[4-(1-methylethyl)phenyl]urea; CAS No. 34123-59-6).

The structure of 3-[4-(2'-hydroxy-2'- propyl)-phenyl]-methyl urea is presented in Figure 1.

Figure 1: Structure of 3-[4-(2'-hydroxy-2'- propyl)-phenyl]-methyl urea



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Isoproturon

Isoproturon is a selective pre- and post-emergence herbicide used to control annual grasses and annual broad leaved weeds in winter and spring wheat, winter and spring barley, winter rye and triticale. Isoproturon is sold worldwide under the trade names Alpha IPU 500; Alpha Isoproturon, Ingot, Koala, Trump, Javelin, Javelin Gold, Protugan and Tolugan Extra (PSD, 1995; Lewis *et al.,* 2007).

Isoproturon is often supplied as a soluble concentrate that is mixed with water and used as a spray (Lewis *et al.,* 2007).

2.2 Environmental fate

2.2.1 Parent

Isoproturon is moderately soluble in water (70.2 mg/L at 20^{o}C). In assessing the sorption to soil, a K_{oc} value of 139 L/ kg has been reported suggesting moderate mobility in the environment (Lewis *et al.,* 2007).

Degradation studies have shown isoproturon to be readily degraded in soils with a half-life of 12 and 23 days under laboratory (20^{o}C) and field conditions respectively. The compound is not considered to be persistent in the environment (Lewis *et al.,* 2007).

2.2.2 Metabolite: 3-[4-(2'-hydroxy-2'- propyl)-phenyl]-methyl urea

3-[4-(2'-hydroxy-2'- propyl)-phenyl]-methyl urea is one of the metabolites formed following degradation of isoproturon.

No information on the physicochemcial properties or environmental fate of 3-[4-(2'-hydroxy-2'- propyl)-phenyl]-methyl urea, was identified.

2.3 Potential routes of human exposure

Exposure of humans to the parent isoproturon may occur through ingestion of contaminated water (oral route), or through occupational handling. Because of isoproturon's low vapour pressure and short half-life in soil, it is unlikely that there is

significant human exposure from air. It is generally considered that diet is not a major source of exposure to isoproturon for the general population. No measurable residues of isoproturon were detected in grain samples, where the detection limit ranged from 0.1 to 0.01 mg/kg (WHO, 1996).

Although no data on routes of exposure of humans to the metabolite desmethylisoproturon was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of isoproturon, or when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of isoproturon in humans.

Once absorbed isoproturon is widely and evenly distributed throughout the body with no accumulation (WHO, 1996).

In a single dose oral study, male and female rats of the RAI strain were given 5.52 or 5.69 mg/kg ^{14}[C]-isoproturon respectively; approximately 90% and 85% of the dose was excreted in urine in males and females, respectively, with 7 and 10% of dose respectively excreted in faeces. A half life of < 8 hr was determined (PSD, 1995). In male rats of the same strain, at a higher dose of 52.6 mg/kg of ^{14}[C]-isoproturon, the half life remained at about 8 hr and routes of excretion were similar (86 and 15% of dose in urine and faeces respectively).

Isoproturon was completely metabolised and largely excreted in the urine; the predominant metabolites are believed to have been formed by oxidation of the isopropyl group and N-demethylation. (PSD,1995).

No information is available on the toxicokinetics of 3-[4-(2'-hydroxy-2'- propyl)-phenyl]-methyl urea in humans.

4 Toxicity Profile

4.1 Isoproturon

4.1.1 Acute Toxicity

No information is available on the acute toxicity of isoproturon in humans.

In rats, isoproturon has moderate acute toxicity; LD_{50}'s are 1826 mg/kg, 2000 mg/kg and 1.95 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis *et al.,* 2007).

Isoproturon is not an irritant to the eye or skin and is not considered a skin sensitizer (WHO, 1996).

4.1.2 Repeat dose toxicity

Principal organs of toxicity following repeated exposure of humans to isoproturon are the red blood cells and liver (EC, 2002).

Data on potential health effects following repeated exposure of humans to isoproturon has been reported on groups of workers involved in its manufacture. However, urine and blood analyses found no effect on peripheral blood count or evidence of haemolytic anaemia (WHO, 1996).

Experimentally, in rats fed diets containing isoproturon at up to 2000 mg/kg diet for two years, increased serum enzyme activities and cholesterol levels (indicative of hepatic enzyme induction) were noted at the highest dose. A marginal reduction in

red blood cell parameters and increase in liver weight and incidence of hepatic acidophilic foci, at histopathology, were reported at the two highest doses. A NOAEL of 80 mg/kg (equivalent to 3.1 and 3.8 mg/kg bw/day) in males and females respectively, was established (WHO, 1996).

4.1.3 Carcinogenicity and mutagenicity

No information on the carcinogenic or mutagenic potential of isoproturon in humans was identified.

In Sprague-Dawley rats fed a diet containing isoproturon at up to 2000 ppm for two years, a decrease in food consumption and associated reduction in body weight gain were noted at the highest dose administered. At termination, higher liver weight was noted at 400 and 2000 ppm. Increased heptocholangiocarcinoma and cholangiocarcinoma were noted in rats of both sexes at 2000 ppm and in males at 400 ppm. Some treated females also developed hepatic haemangiosarcomas, a rare tumour, and this was considered to represent a treatment-related effect. At all doses investigated, males showed increased foci or areas of hepatic alteration; similar changes were also seen in females at 400 or 2000 ppm (PSD 1995). A NOAEL of 80 ppm (3.1 mg/kg bw/day) was established (EU, 2002).

Experimentally, isoproturon has been tested in *in vitro* and *in vivo* assays for mutagenicity. No evidence of mutagenicity has been demonstrated in bacterial, eukaryotic or *in vitro* and *in vivo* mammalian cells (WHO, 1996).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of isoproturon in humans was identified.

In a two-generation study in Wistar rats, isoproturon was administered in the diet at levels up to 400 ppm. F_0 males were treated for 10 weeks and females for 12 weeks prior to mating. Treatment was continuous until weaning of F_2 offspring. No effects were seen in F_0 animals. Reduced body weight gain was noted in F_1 parents and offspring at 400 ppm. Retarded spermatogenesis was seen in two F_1 males at 400 ppm and one F1 male at 200 ppm. Some males at 400 ppm also showed focal hyperplasia in the seminal vesicles and prostate. At the highest dose, a slight reduction in pregnancy rate was seen in F_1 animals. A reproductive NOAEL of 100 ppm (10 mg/kg/day) was established (PSD, 1995; WHO, 1996).

Teratology studies for isoproturon have been undertaken in female New Zealand White rabbits given isoproturon by oral gavage at up to 160 mg/kg/day from day 6 to day 18 of gestation. Maternal food consumption and body weight gain were reduced at the highest dose at which an increased number of runts (fetuses weighing < 70% of mean litter weight) was noted. Although the number of fetuses per dam was higher at the highest dose, this was associated with lower placental and fetal weights, and changes were not attributed to treatment. A developmental NOEL of 40 mg/kg/day was established (PSD, 1995, WHO, 1996).

4.2 3-[4-(2'-hydroxy-2'- propyl)-phenyl]-methyl urea

No information on the repeat dose toxicity of 3-[4-(2'-hydroxy-2'- propyl)-phenyl]-methyl urea in humans was identified.

Searches were made for publically available information on the toxicity of 3-[4-(2'-hydroxy-2'- propyl)-phenyl]-methyl urea, including use of the online programme

ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK did not identify the presence of any structural alerts.

The analysis by TOPKAT suggested that 3-[4-(2'-hydroxy-2'- propyl)-phenyl]-methyl urea was:

carcinogenic; but
non-mutagenic.

Prediction of the reproductive and developmental toxicity of 3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea using TOPKAT was not possible.

Table 1: Predicted toxicity data for 3-[4-(2'-hydroxy-2'- propyl)-phenyl]-methyl urea using DEREK and TOPKAT software

Compound (data source)	**Acute toxicity**		**Repeat dose toxicity**	**Genotoxicity / Mutagenicity**	**Carcinogenicity**	**Reproductive & Developmental toxicity**
	LD50 mg/kg (range)	**LC50 mg/m^3/H**				
Isoproturon (experimental data)	1826	1.95	NOAEL: 3.1 mg/kg/day Main target organs: Red blood cells and liver	Negative	Hepatocellular tumours and cholangiocarcin-omas in rats.	Evidence of retarded spermatogenesis and decreased pregnancy rate (rats). Increased number of runts (rabbits).
3-[4-(2'-hydroxy-2'- propyl)-phenyl]-methyl urea (TOPKAT)	947.7 (222.3-4000)	413.6 (45.7-3700)	MTD (feed/drink) 117.2 mg/kg MTD (oral gavage) 117.2 mg/kg	Negative	Positive	UE
3-[4-(2'-hydroxy-2'- propyl)-phenyl]-methyl urea (DEREK)	n/a	n/a	No alert	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software; UE- unreliable estimate

5 Guidelines and Standards

5.1 Isoproturon

The EC risk classification is: Carcinogen category 3: R40; N - Dangerous for the environment: R50, R53 and EU safety category is S2, S36/37, S60, S61. Isoproturon is classified by WHO as 'slightly hazardous' (Lewis *et al*., 2007).

An ADI of 0.015 mg/kg bw/day has been established (EU, 2002) for isoproturon using a safety factor (SF) of 200, based on a two year rat study in which Sprague-Dawley rats received a diet containing isoproturon at up to 2000 ppm Lewis *et al*., 2007). It should be noted however, that full study details were not available to fully assess the robustness of the SF applied in the study.

5.2 3-[4-(2'-hydroxy-2'- propyl)-phenyl]-methyl urea

Due to the lack of availability of experimental data, it has not been possible to establish a robust NOEL or NOAEL for 3-[4-(2'-hydroxy-2'- propyl)-phenyl]-methyl urea, and no ADI has been proposed by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

Although apparently likely to retain the carcinogenic potential of the parent, the toxicity profile for

3-[4-(2'-Hydroxy-2'- propyl)-phenyl]-methyl urea is predicted by TOPKAT but not DEREK to retain the carcinogenic potential of the parent compound. However, both the acute oral toxicity and oral MTD predicted by TOPKAT are substantially higher than the comparable values for the parent. Thus, the predicted pattern of toxicity for this metabolite is substantially different than that for the other metabolite of isoproturon, desmethylisoproturon, considered in Appendix 11.15.

The highest oral MTD predicted for 3-[4-(2'-Hydroxy-2'- propyl)-phenyl]-methyl urea by TOPKAT is 117.2 mg/kg (irrespective of route of administration). Due to the predicted carcinogenicity, it is considered appropriate to apply a SF of 1000, giving a nominal value of 0.117 mg/kg bw/day, which is above the ADI of 0.015 mg/kg bw/day established for the parent isoproturon. Given this, it is therefore proposed to adopt a PSDV of 0.015 mg/kg bw/day for desmethylisoproturon (i.e. the same value as for the parent). Based on the oral MTD predicted by TOPKAT, this will provide an overall SF of >1000.

6 References

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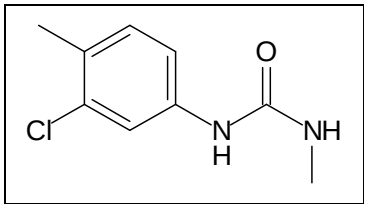
Appendix 12.20 3-(3-chloro-p-tolyl)-1-methylurea

1 Introduction

3-(3-Chloro-p-tolyl)-1-methylurea is a major metabolite of the herbicide chlorotoluron (*N*'-(3-chloro-4-methylphenyl)-*N*,*N*-dimethylurea; CAS No. 15545-48-9) and is formed within the plant and surrounding soil (PSD, 1995). The metabolite is also formed during the metabolism of ingested chlorotoluron in humans.

The structure of 3-(3-chloro-p-tolyl)-1-methylurea, is presented in Figure 1.

Figure 1: Structure of 3-(3-chloro-p-tolyl)-1-methylurea



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Chlorotoluron

Chlorotoluron is a selective, non-systemic herbicide that is used pre- or early post-emergence to control annual grasses and broad-leaved weeds in winter cereals (WHO, 1996). Chlorotoluron is sold in Europe under the commercial names Tolerate, Alpha Chlorotoluron 500, Tolugan and Tolugan Extra. It is usually supplied as a soluble concentrate or wettable granules that are mixed with water and supplied as a spray (Lewis *et al*., 2007).

2.2 Environmental fate

2.2.1 Parent

Chlorotluron is moderately soluble in water (74 mg/L at 20^{o}C). In assessing the sorption to soil, a K_{oc} value in the range 205 L kg^{-1} has been reported suggesting moderate mobility in the environment (Lewis *et al*., 2007).

Chlorotoluron is degraded in soils and half-lives of 59 and 34 days have been estimated under laboratory (20^{o}C) and field conditions respectively. The compound is considered to be moderately persistent in the environment (Lewis *et al*., 2007).

2.2.2 Metabolite: 3-(3-chloro-p-tolyl)-1-methylurea

3-(3-Chloro-p-tolyl)-1-methylurea is one of the major metabolites formed following degradation of chlorotoluron, with an estimated maximum formation fraction of 0.300 (30%; Lewis *et al*., 2007).

No information on the physicochemical properties or environmental fate of 3-(3-chloro-p-tolyl)-1-methylurea was identified.

2.3 Potential routes of human exposure

During its production and use, chlortoluron may be directly released into the environment. If released to the atmosphere, chlortoluron will exist solely in the particulate phase which may be physically removed from the air by wet and dry deposition (HSDB, 2002).

Exposure of humans to the parent chlorotoluron may occur through ingestion of contaminated food and water or via inhalation or dermal contact during occupational handling or bystander exposure (HSDB, 2002).

Although no data on routes of exposure of humans to the metabolite 3-(3-chloro-p-tolyl)-1-methylurea was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of chlorotoluron, or when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of chlorotoluron in humans.

In rats, chlorotoluron has been shown to be readily absorbed from the GI tract; 75% of radiolabelled compound being absorbed following oral dosing (EU, 2005; unpublished company report).

Tolyl-^{14}[C]-chlorotoluron administered to rats in a two week feeding study was widely distributed. Highest activity was detected in liver, spleen and kidneys within nine days (Kearney and Kaufman, 1975).

In a study in which ^{14}[C]-chlorotoluron was given as a single oral gavage to rats, it was rapidly and efficiently excreted. The principal route of excretion was via urine (around 80%; Kearney and Kaufman, 1975).

The initial stages of metabolism in rats involved substitution of the methyl ring and subsequent stepwise oxidation yielding the corresponding hydroxymethyl and then carboxy derivative. Of 11 urinary metabolites isolated, nine were identified as: 3-(3-chloro-4-methylthiomethylphenyl)-1,1-dimethylurea; 3-(3-chloro-4-methylthiomethylphenyl)-1-methylurea; (3-chloro-4-methylthiomethylphenyl)urea; (3-chloro-4-methylphenyl)urea; 3-(3-chloro-4-hydroxymethylphenyl)-1,1-dimethylurea, free and conjugated; 3-(3-chloro-4-hydroxymethylphenyl)-1-methylurea; (3-chloro-4-hydroxymethylphenyl)urea; 3-(3-chloro-4-carboxyphenyl)-1,1-dimethylurea; 3-(3-chloro-4-carboxyphenyl)-1-methylurea; and (3-chloro-4-carboxyphenyl)urea (Kearney and Kaufman, 1975).

No information on the toxicokinetics of 3-(3-chloro-p-tolyl)-1-methylurea acid was identified.

4 Toxicity Profile

4.1 Chlorotoluron

4.1.1 Acute Toxicity

The acute effects of chlorotoluron in humans is unknown.

In rats, chlorotoluron has low acute toxicity; LD_{50}'s are 10,000 mg/kg, 2000 mg/kg and 5.3 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis *et al*., 2007).

Chlorotoluron is not an irritant to the eye or skin. There is no evidence of skin sensitisation with chlorotoluron (EU, 2005; unpublished company report).

4.1.2 Repeat dose toxicity

No information regarding the effects of repeated exposure to chlorotoluron in humans was found.

Experimentally, the principal target organ of repeated exposure to chlorotoluron is the liver in rats and the kidneys in mice. In 90-day oral feeding studies in rats and dogs,

NOELs of 53 mg/kg/day in rats and 23 mg/kg/day in dogs were established (HSDB, 2002; full study details unavailable).

A NOAEL of 100 ppm (equivalent to 3.7 mg/kg bw/day) was reported in a two-year chlorotoluron feeding study in male rats (EU, 2005; unpublished company report).

4.1.3 Carcinogenicity and mutagenicity
No information on the potential carcinogenic and mutagenic effects of chlorotoluron in humans was identified.

Carcinogenic potential has been demonstrated in a 2-year feeding study in male mice and rats administered chlorotoluron at doses up to 100 ppm (3.7 mg/kg bw/day). An increased incidence of kidney adenoma and adenocarcinoma was reported in mice; however chlorotoluron was not carcinogenic in Sprague-Dawley rats (EU, 2005; unpublished company report). Experimentally, no conclusive evidence of genotoxic potential has been demonstrated for chlorotoluron (EU, 2005; unpublished company report).

4.1.4 Reproductive and development toxicity
No information was found on the reproductive and developmental potential of chlorotoluron to humans.

Experimentally, in a two-generation study in rats, chlorotoluron resulted in increased resorption rate but only at parentally toxic doses. A NOAEL of 1000 ppm (equivalent to 95 mg/kg bw/day) was established for reproductive endpoints (EU, 2005; unpublished company report). Fetotoxicy (reduced mean weight and skeletal anomalies) was also noted at maternal toxic doses. Increased fetal resorption and skeletal anomalies were also noted at maternally toxic doses in rabbits. A NOAEL of 50 mg/kg bw/day was established for developmental endpoints (EU, 2005; unpublished company report).

4.2 3-(3-Chloro-p-tolyl)-1-methylurea acid
No information on the repeat dose toxicity of 3-(3-chloro-p-tolyl)-1-methylurea acid in humans was identified.

Searches were made for published information on the toxicity of 3-(3-chloro-p-tolyl)-1-methylurea acid, including use of the online programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.

Additional information was, therefore, sought through application of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Summaries of the toxicity data predicted for 3-(3-chloro-p-tolyl)-1-methylurea acid from these tools are summarised below (Table 1).

DEREK identified no structural alerts in the molecule.

The analysis by TOPKAT suggested that 3-(3-chloro-p-tolyl)-1-methylurea acid was:

carcinogenic; but
non-mutagenic; and
a developmental toxicant.

Table 1: Predicted toxicity data for 3-(3-chloro-p-tolyl)-1-methylurea acid using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Chlorotoluron (experimental data)	10,000	5.3	NOAEL 3.7 mg/kg bw/day Main target organs: Liver (rats) and Kidney (mice)	Genotoxic potential not established	Kidney carcinogen (mice)	Increased incidence of resorptions (rats and rabbits). Reduced foetal weight and increased skeletal anomalies (rats and rabbits)
3-(3-chloro-p-tolyl)-1-methylurea acid (TOPKAT)	947.7 (222.3-4000)	413.6 (45.7-3700)	MTD (feed/drink) 117.2 mg/kg bw/day MTD (oral gavage) 117.2 mg/kg bw/day	Negative	Positive	Developmental Toxicant
3-(3-chloro-p-tolyl)-1-methylurea acid (DEREK)	n/a	n/a	No alert	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software

5 Guidelines and Standards

5.1 Chlorotoluron

The EC risk classification is: Carcinogen category 3: R40; Xn – Harmful: R63; N – Dangerous for the environment: R50, R53. The EC safety classification is S2, S26, S36/37, S45, S60, S61.Chlorotoluron is classified by WHO as 'unlikely to present acute hazard in normal use' (Lewis *et al*., 2007).

An ADI of 0.04 mg/kg bw/day has been established for chlorotoluron based on a two year rat feeding study (EU, 2005; unpublished company report) using a safety factor (SF) of 100 (Lewis *et al*., 2007).

5.2 3-(3-chloro-p-tolyl)-1-methylurea acid

Due to the lack of availability of experimental data, it has not been possible to establish a robust NOEL or NOAEL for 3-(3-chloro-p-tolyl)-1-methylurea, and no ADI has been proposed by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

Although apparently likely to retain the carcinogenic potential and developmental toxicity of the parent, the toxicity profile for 3-(3-chloro-p-tolyl)-1-methylurea acid is overall predicted as somewhat less than that of the parent. The oral MTD predicted by TOPKAT for 3-(3-chloro-p-tolyl)-1-methylurea acid is 117.2 mg/kg bw (irrespective

of nature of oral administration). Applying a SF of 100 would give a nominal value of 1.1 mg/kg bw, which would be significantly above the established ADI of 0.04 mg/kg/bw for the parent chlorotoluron. Given this, it is – on a highly precautionary basis (to allow for the predicted carcinogenic and developmental potential) – therefore proposed to adopt a PSDV of 0.04 mg/kg bw/day for 3-(3-chloro-p-tolyl)-1-methylurea acid, i.e. the same value as for the parent; this would provide an overall SF of approx. 2900.

6 References

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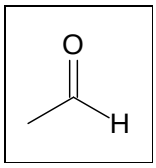
Appendix 12.21 Acetaldehyde

1 Introduction

Acetaldehyde (CAS No. 75-07-0) is a metabolite of the molluscicide metaldehyde (2,4,6,8-tetramethyl-1,3,5,7-tetraoxacyclooctane; CAS No. 108-62-3) that is formed within the surrounding soil following application of the parent compound. It is also formed during the metabolism of absorbed metaldehyde in humans.

The structure of acetaldehyde, is presented in Figure 1.

Figure 1: Structure of acetaldehyde



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Metaldehyde

Metaldehyde is a molluscicide that is used as a slug and snail poison. It is approved for use in agriculture, horticulture and the home garden. Over 80% of its agricultural use is with oilseed rape, wheat, winter barley and potatoes (PSD, 1986).
Metaldehyde is currently sold worldwide under several commercial names including Certis Deal 5, Meridien, Carakol and Metalden. It is generally sold as ready-to-use bait (Lewis *et al*., 2007).

2.2 Environmental fate

2.2.1 Parent

Metaldehyde is moderately soluble in water (188 mg/L) and, in assessing the sorption to soil, a K_{oc} value of 85 L kg^{-1} has been reported suggesting moderate mobility in the environment (Lewis *et al*., 2007).

Metaldehyde is moderately susceptible to degradation in soil under aerobic conditions with a degradation half-life of 11.9 days when measured under laboratory conditions (20^{o}C); metaldehyde is considered to be non-persistent (Lewis *et al*., 2007).

Acetaldehyde is one of the metabolites formed following degradation of metaldehyde, with an estimated maximum formation fraction of 0.05 (5%; Lewis *et al*., 2007).

2.2.2 Metabolite: acetaldehyde

Acetaldehyde is highly soluble in water (356800 mg/L) and, in assessing the sorption to soil, a K_{oc} value of 1.5 L kg^{-1} has been reported (Lewis *et al*., 2007) suggesting high mobility in the environment.

No further information relating to either the physicochemical properties or environmental fate of acetaldehyde could be found.

2.3 Potential routes of human exposure

Exposure to metaldehyde may occur through ingestion of contaminated food and water or as a result of dermal contact during occupational handling (HSDB, 2003).

Although no data on routes of exposure of humans to the metabolite acetaldehyde was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of metaldehyde, or when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics of parent and metabolite

3.1 Metaldehyde

No information is available on the toxicokinetics of metaldehyde in humans.

A toxicokinetic study on metaldehyde has been carried out in rats given a single oral gavage of 14[C]-metaldehyde at 9.8-10.5 mg/kg bw or 102 mg/kg bw. In males, levels of radiolabel peaked at between one and two - hours after dosing and a half-life of 3.4 hrs was calculated. In females, peak radiolabel was slightly delayed occurring after two to four hours and the half-life was 8.8 hours (PSD, 1986).

Although most of the residual radioactivity occurred in the carcass and the GI tract and its contents, metaldehyde was also widely distributed (amounting to 7.3 - 10.7% of administered dose; PSD, 1986). Both metaldehyde and acetaldehyde (but no other metabolites) were present in blood. The majority of radioactivity was excreted in urine, faeces and expired air (recoveries were 89 - 98%, 2.6 - 3.4% and 2.5 - 2.8% in males, and 78 - 93%, 4.2 - 5.1% and 2.7 - 2.8% in females, respectively; PSD, 1986).

The parent compound did not occur in urine but numerous metabolites were present (PSD, 1986).

3.2 Acetaldehyde

In humans, acetaldehyde is rapidly absorbed and is completely metabolized, mostly by the liver. Up to < 5% may be excreted in expired air; acetaldehyde is not however itself excreted in the urine (HSDB, 2005) but is further metabolised to N-nitroso-2-methylthiazolidine 4-carboxylic acid which has been detected in the urine of human subjects following oral and inhalation exposures (ACGIH, 1991).

4 Toxicity Profile

4.1 Metaldehyde

4.1.1 Acute Toxicity

In humans, acute oral ingestion of metaldehyde may result in vomiting, seizure, increased temperature and memory loss; fatalities have been reported (doses unknown; HSDB, 2003).

Experimentally, metaldehyde has moderate acute toxicity in rats; LD_{50}'s are 283 mg/kg, 5000 mg/kg and 15 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis *et al*., 2007).

Metaldehyde is not a contact irritant to the skin or eye in animals and no evidence of skin sensitisation has been detected (PSD, 1986).

4.1.2 Repeat dose toxicity

No information on the effects of repeated exposure of metaldehyde to humans was found.

In Sprague-Dawley CD rats given diets containing metaldehyde at levels up to 5000 ppm for two years, reduced body weight gain in the absence of altered food consumption was noted at the two highest doses (1000 and 5000 ppm) . No treatment related effects were reported on haematology or urinalysis. Clinical

chemistry changes in males were unremarkable but, in females, serum cholesterol levels were noted at the two highest doses. At 5000 ppm, absolute and body weight relative liver weights were increased in both sexes although the effect was most marked in females. Histopathologically, a dose-related increase in incidence and severity of hepatocellular hypertrophy was noted in both sexes at 1000 or 5000 ppm; a NOEL of 50 ppm (2.5 mg/kg bw/day was established) (PSD, 1986).

4.1.3 Carcinogenicity and genotoxicity
No information on the carcinogenic or mutagenic potential of metaldehyde in humans was found.

In the two year rat dietary study described above, despite the non-neoplastic liver pathology, the incidence of hepatocellular adenoma in males and hepatocellular carcinoma in males and females was not affected by treatment. A significant increase in hepatocellular adenoma was reported in females at 5000 ppm, however, when compared with historical control data, this was not considered to be treatment related. A NOEL of 50 ppm (equivalent to 2.5 mg/kg bw/day) was established for carcinogenic effects.

Experimentally, no evidence of mutagenicity has been shown in *in vitro* assays using five strains of *S. typhimurium* with or without metabolic activation or in mouse lymphocytes. Metaldehyde is not considered to be mutagenic or genotoxic (PSD, 1986).

4.1.4 Reproductive and development toxicity
No information was identified on the reproductive or developmental effects of metaldehyde in humans.

In a multi-generation reproduction study in Wistar rats, metaldehyde dietary administration at up to 5000 ppm resulted in a high mortality rate amongst dams, and in hind-limb paralysis in animals of all generations at the highest dose paralysis correlated with histopathologically apparent transverse lesions of the spinal cord. In the F2 generation, depressed growth was also noted at 5000 ppm. After seven months exposure to metaldehyde, a dose-related increase in the activities of the liver enzymes amidopyrindemethylase (APDM) and alcohol dehydrogenase (ADH) were observed in both the F1 and F2 generations; an increase in mean body weight-relative liver weight was also noted in F1 dams and F2 males at 5000 ppm. Bodyweight-relative mean thyroid weight was also increased in F0 males and F2 males and females but was decreased in F1 females at 5000 ppm. Fertility rates were lower in males of the 1000 or 5000 ppm groups and in females fed 5000 ppm. Viability and lactation indices were also decreased in F1 and F2 animals fed 5000 ppm. Microscopic examination of fetuses showed no treatment-related developmental abnormalities. A NOEL for reproductive effects of 200 ppm (10 mg/kg bw/day) was established based on reduced fertility viability and lactation indices. (PSD, 1986).

4.2 Acetaldehyde
4.2.1 Acute Toxicity
Acute i.v. route exposure of acetaldehyde to humans resulted in increased heart rate, and pulmonary ventilation and dead space and a fall in alveolar carbon dioxide level (IARC, 1985).

Acetaldehyde has moderate acute toxicity in rats; the oral LD_{50} is 661 mg/kg (Lewis *et al*., 2007).

In animals, acetaldehyde is irritant to the skin, eye and respiratory system but no evidence of skin sensitisation has been shown (HSDB, 2005).

4.2.2 Repeat dose toxicity

No information was found on the effects of repeated exposure to acetaldehyde in humans.

Experimentally, in Wistar rats exposed for 28 months for six hours per /day, five days/wk, to acetaldehyde at 1350, 2700 or 1800 - 5400 mg/m^3 (high dose was reduced to 1800 mg/m^3 from week 20), increased mortality, growth retardation and generative changes of the olfactory and nasal epithelia were noted in all treated groups. Other histopathological changes included slight to severe hyperplasia and, the presence of keratinized stratified metaplasia of the larynx (in the high dose only) and generative changes of the upper respiratory epithelium (including papillomatous hyperplasia at the high dose). In the trachea, there was focal flattening and irregular arrangement of the epithelium in 3/10 high-dose males (WHO, 1995).

The hepatotoxic potential of acetaldehyde was assessed in male Wistar rats given oral doses of between 17.9 and 35.8 mmol/kg or i.p. doses of between 4.5 and 9 mmol/kg. Acetaldehyde was shown not to affect enzyme activities, triglyceride levels or to cause histologic changes when compared to controls (Strubelt et al., 1987).

4.2.3 Carcinogenicity and mutagenicity

There is inadequate evidence for the carcinogenicity of acetaldehyde in humans (HSDB, 2005).

In the 28 month rat study reported above, nasal carcinoma was seen in all treated groups. Although tumour incidence was dose-related, the latency period appeared independent of concentration with the first tumours appearing within twelve months of treatment starting in all groups. The incidences of tumours of the lungs, larynx and trachea were not affected by treatment (WHO, 1995).

The incidence of respiratory tract tumours was further assessed in Syrian golden hamsters exposed for seven hours per day, five days per week for 52 weeks to acetaldehyde at gradually decreasing concentrations of between 2500 and 1650 ppm. Increased tumour incidences were reported in males and females, predominantly in the form of laryngeal carcinomas and a few laryngeal polyps and nasal polyps & carcinomas (IARC, 1985).

Acetaldehyde was not mutagenic in several strains of *S. tryphimurium* (with and without metabolic activation). It was also non-mutagenic in *E. coli* and *Drosophila melanogaster*. However, in mammalian *in vitro* assays, acetaldehyde caused dose-related increases in the levels of sister chromatid exchange and of chromosomal break and aberrations in Chinese hamster ovary cell cultures. In *vivo* assays, acetaldehyde produced chromosomal aberrations in rats and sister chromatid exchanges in mice and hamsters (IARC, 1999).

Overall, IARC considered that there was sufficient evidence in experimental animals to support the carcinogenicity of Acetaldehyde and concluded that the compound was '*possibly carcinogenic to humans*' (Group 2B; IARC, 1999).

4.2.4 Reproductive and developmental toxicity

No information on the reproductive and developmental effects of acetaldehyde in humans was identified.

In Fischer rats given acetaldehyde orally at 240 mg/kg bw/day from the first day of pregnancy through to the end of lactation, maternal body weight gain between the first and 20th day of pregnancy was lower in treated animals compared with the control group. Placental weights were also reduced in mothers administered acetaldehyde. Histologically brain, liver and kidney showed slight changes in all treated dams compared with a control group that showed almost no abnormalities. The average number of fetuses on gestation day 20 and number of neonates per litter at birth were unaffected by acetaldehyde, however, offspring body weight was reduced by treatment. Histological examination of the brain, lung, liver, kidney and thymus of offspring showed treatment-related visceral immaturity and haemorrhage (HSDB, 2005).

In a developmental toxicity study in which CF rats were given acetaldehyde at 50 mg/kg bw/day i.p. on days 8 through 15 of gestation, fetuses showed significant delays in ossification and skeletal malformations (e.g. wavy ribs; Sreenathan *et al.,* 1984).

Additional information was sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Analysis by DEREK identified a number of structural alerts in the molecule leading to the conclusions that:

it is considered plausible (i.e. there is a weight of evidence) that acetaldehyde will cause skin sensitisation in humans. This endpoint is predicted because acetaldehyde is an aldehyde.

it is considered plausible (i.e. there is a weight of evidence) that acetaldehyde will cause mutagenicity in humans. This endpoint is predicted because acetaldehyde is an alkyl aldehyde or precursor.

it is considered plausible (i.e. there is a weight of evidence) that acetaldehyde will cause genotoxicity in humans. This endpoint is predicted because acetaldehyde is an alkyl aldehyde or precursor.

it is considered plausible (i.e. there is a weight of evidence) that acetaldehyde will cause chromosome damage in humans. This endpoint is predicted because acetaldehyde is an alkyl aldehyde or precursor.

The analysis by TOPKAT suggested that acetaldehyde was:

carcinogenic; but
non-mutagenic.

Prediction of the reproductive and developmental toxicity of acetaldehyde using TOPKAT was not possible
.

Table 1: Predicted toxicity data for acetaldehyde using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Metaldehyde (experimental data)	283	15	NOAEL: 2.5 mg/kg/day Main target organ: liver	Negative	Negative	Reduced fertility viability and lactation indices.
Acetaldehyde (experimental data)	661	n/d	Upper respiratory tract changes	Negative	Increased incidence of laryngeal tumours	Reduced body weight of offspring; delay in ossification and increased skeletal malformations.
Acetaldehyde (TOPKAT)	476.4 (132.7-1700)	10,000 (771.8-10,000)	MTD (feed/drink) 91.7 mg/kg MTD (oral gavage) 253.3 mg/kg	Negative	Positive	Indeterminate
Acetaldehyde (DEREK)	n/a	n/a	*Plausible* for skin sensitisation in humans	*Plausible* in humans	*Plausible* for chromosome damage and *mutagenicity* in humans	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software

5 Guidelines and Standards

5.1 Metaldehyde

The EC risk classification for metaldehyde is: Xn – Harmful: R22; H - Handling risks: R11.EC safety classification for metaldehyde is S2, S13, S16, S25, S46. Metaldehyde is classified by WHO as 'moderately hazardous', and by the US EPA as 'moderately toxic' (Lewis *et al*., 2007).

An ADI of 0.02 mg/kg bw/day has been established for metaldehyde (PSD, 1986) based on a NOAEL of 2.5 mg/kg bw/day , derived from a two year rat study in which Sprague-Dawley rats received metaldehyde via the diet at up to 5000 ppm; a safety factor (SF) of 100 applied (Lewis *et al*., 2007).

5.2 Acetaldehyde

The EC risk classification for acetaldehyde is Carcinogen category 3: R40; H - Handling: R12; Xi - Irritant: R36, R37. EC safety classification for acetaldehyde is S2, S16, S33, S36/37. Acetaldehyde is not listed under WHO or US EPA classifications.
No ADI has been proposed by any authoritative organisation for acetaldehyde. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The available experimental data, supported by the predicted software, suggest that the metabolite is likely to retain many of the toxic properties of the parent. However, these predictive systems also suggest that the repeated (but not acute) toxic activity of acetaldehyde may be somewhat less than that of the parent. This is apparent from the lowest oral MTD value of 91.7 mg/kg bw/day predicted by TOPKAT (for diet or drinking water administration) for acetaldehyde. Applying a SF of 100 would give a nominal value of 0.92 mg/kg bw which is significantly above the established ADI of 0.02 mg/kg/bw for the parent compound, metaldehyde. Given this, it is – on a highly precautionary basis – therefore proposed to adopt a PSDV of 0.02 mg/kg bw/day for acetaldehyde, i.e. the same value as for the parent; this will provide an overall SF of 4585.

6 References

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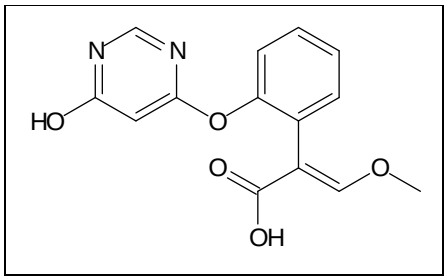
Appendix 12.22 Reference Compound 10

1 Introduction

Reference compound 10 is a metabolite of the fungicide azoxystrobin (methyl (α*E*)-2-[[6-(2-cyanophenoxy)-4-pyrimidinyl]oxy]-α-(methoxymethylene)benzeneacetate; CAS No. 131860-33-8) and is formed within the surrounding soil and during the metabolism of ingested azoxystrobin by humans.

The structure of reference compound 10 is presented in Figure 1.

Figure 1: Structure of reference compound 10



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Azoxystrobin

Azoxystrobin is a fungicide that is widely used in farming, particularly to protect plants and fruit/vegetables from fungal disease.

Azoxystrobin is currently sold worldwide under several commercial names including: Amistar; Amistar Opti; Amistar Pro; Olympus; Priori Xtra; Quadris; and Abound. It is generally sold as a concentrated suspension that is diluted for spray application (Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Use of azoxystrobin will result in its direct release to the environment where it will exist solely in the particulate phase of ambient air. Particulate-phase azoxystrobin will be removed from the atmosphere by wet and dry deposition.

Azoxystrobin has low solubility in water (6.7 mg/L) and, in assessing the sorption to soil, a Koc value of 423 L kg-1 has been described suggesting moderate mobility in the environment (Lewis et al., 2007).

Azoxystrobin is moderately susceptible to degradation in soil under aerobic conditions, with a typical degradation half-life of 70 days. Azoxystrobin is considered to be moderately persistent (Lewis et al., 2007).

2.2.2 Metabolite: Reference compound 10

Reference compound 10 is one of the metabolites formed during metabolism or degradation of azoxystrobin.

No information on the physiochemical properties or environmental fate of reference compound 10 was identified.

2.3 Potential routes of human exposure

Exposure to azoxystrobin may occur through inhalation of dust and dermal contact at places where it is produced or used as a fungicide and through ingestion of contaminated food and water (HSDB, 2002).

Although no data on routes of exposure of humans to the metabolite reference compound 10 was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of azoxystrobin, or when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics of parent and metabolite

3.1 Azoxystrobin

No information is available on the toxicokinetics of azoxystrobin in humans.

Metabolism studies using both labelled and un-labelled azoxystrobin given orally to lactating goats as single doses or for up to 14 days. Absorption is dose-dependent, amounting to nearly 100% at low doses. Following absorption, it is widely distributed; highest levels occur in the kidneys and liver but there is no evidence of bioaccumulation. Azoxystrobin is extensively metabolised with at least 18 metabolites detected. The metabolic pathway is believed to involve hydrolysis and subsequent glucuronide conjugation. Excretion is via the faeces (73 - 89%) and urine (9 - 18%); biliary excretion accounts for < 10% of administered dose (Webb et al., 1996; US EPA, 2000).

3.2 Reference compound 10

No information on the toxicokinetics of reference compound 10 was found.

4 Toxicity Profile

4.1 Azoxystrobin

4.1.1 Acute Toxicity

The acute toxicity of azoxystrobin to humans is not known.

Experimentally, azoxystrobin has low acute toxicity in rats; LD50's are 5000 mg/kg, 2000 mg/kg and 0.69 mg/m3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007).

In animals azoxystrobin is not a contact irritant to the skin or eye and there is no evidence of skin sensitisation (EU, 1998).

4.1.2 Repeat dose toxicity

No information relating on the effect of repeated exposure of humans to azoxystrobin was found.

Experimentally, the principal target organs were the liver and common bile duct in male and female rats given azoxystrobin in the diet for two years. In males, reduced body weights, food consumption and food efficiency and increased incidence of bile duct lesions were seen at ≥ 34 mg/kg/day. In females, reduced body weight was noted at ≥ 117.1 mg/kg/day. A NOAEL of 18.2 and 22.3 mg/kg/day was established, for males and females respectively (US EPA, 2000).

4.1.3 Carcinogenicity and genotoxicity

The carcinogenic or mutagenic potential of azoxystrobin in humans is not known and there is no experimental carcinogenicity data available.

No overall conclusions can be reached as to the genotoxicity of azoxystrobin; weak clastrogenic effects have been reported in in vitro assays, but not in in vivo studies (EU, 1998; US EPA, 2000).

4.1.4 Reproductive and development toxicity
The reproductive and developmental toxicity potential of azoxystrobin in humans is unknown.

In the rat feeding study described above, effects on reproduction and fertility were assessed over 2 generations. Reduced pup body weights were observed in both F1 and F2 pups and a NOAEL of 18.2 and 22.3 mg/kg/day were established for males and females respectively.

In a pre-natal developmental study in rats, azoxystrobin administered via the diet resulted in minor skeletal developmental anomalies at maternal toxic doses. A NOAEL of 25 mg/kg/day was established for developmental effects (EU, 1998).

4.2 Reference compound 10
4.2.1 Acute Toxicity
No information on the acute toxicity of reference compound 10 is known.

4.2.2 Repeat dose toxicity
No information was found on the effects following repeated exposure to reference compound 10 in humans.

Searches were made for published information on the toxicity of reference compound 10, including use of the online programme ChemIDPlus (US NLM, 2003). However, no relevant information was found. Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology).Results are summarised below (Table 1).

DEREK identified the presence of two structural alerts and the following conclusions were drawn:

- it is considered plausible (i.e. there is a weight of evidence) that reference compound 10 will cause skin sensitisation in humans. This endpoint is predicted because reference compound 10 is an enol ether.

- it is considered plausible (i.e. there is a weight of evidence) that reference compound 10 will cause carcinogenicity in humans. This endpoint is predicted because reference compound 10 is substituted pyrimidine or purine compound.

The analysis by TOPKAT suggested that reference compound 10 was:

- non-carcinogenic;
- non-mutagenic; but
- a developmental toxicant

Table 1: Predicted toxicity data for Reference compound 10 using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H				
Azoxystrobin (experimental data)	5000	0.69	NOAEL: 18.2 mg/kg/day Main target organ: Liver and common bile duct	Negative	Not defined	Minor reduction in pup body weights; minor effects on skeletal development
Reference compound 10 (TOPKAT)	847.9 (122-5900)	10,000 (10,000-10,000)	MTD (feed/drink) 9 mg/kg MTD (oral gavage) 24.9 mg/kg	Negative	Negative	Developmental toxicant
Reference Compound 10 (DEREK)	n/a	n/a	*Plausible* for skin sensitisation in humans	No alert	*Plausible* in humans	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software

5 Guidelines and Standards

5.1 Azoxystrobin

The EC risk classification is: T – Toxic: R23; N – Dangerous for the environment: R50, R53. Azoxystrobin is classified by WHO as 'unlikely to present acute hazard in normal use'; there is no US EPA classification (Lewis *et al*., 2007).

An ADI of 0.1 mg/kg bw/day has been established for azoxystrobin based on a chronic rat feeding study; a safety factor (SF) of 100 was applied (Lewis *et al*., 2007).

5.2 Reference compound 10

No ADI has been proposed by any authoritative organisation for Reference compound 10. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

It is predicted by TOPKAT, but not DEREK, that reference compound 10 is likely to retain some of the developmental toxicant properties of the parent. However, DEREK suggests a possible carcinogenic activity for the metabolite; this is not predicted by TOPKAT and the carcinogenic potential of the parent azoxystorbin has not been defind experimentally. Overall, the repeat dose toxicity profile for reference compound 10 is similar to that of the parent ,with the lowest oral MTD predicted by TOPKAT for reference compound 10 being 9 mg/kg bw/day (for dietary or drinking water administration). Applying a SF of 100 would give a nominal value of 0.09 mg/kg bw/day. This is similar to the established ADI of 0.1 mg/kg bw/day for the parent

azoxystrobin. Given this, it is proposed to adopt a PSDV of 0.1 mg/kg bw/d for reference compound 10; this gives an overall SF of 90.

6 References

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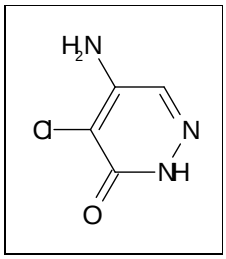
Appendix 12.23 5-Amino-4-chloro-3-(2H)-pyridazinone

1 Introduction

5-Amino-4-chloro-3-(2H)-pyridazinone is a major metabolite of the herbicide chloridazon (5-amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone; CAS No. 1698-60-8) and is formed within surrounding soil following application of the parent to plants. It is also formed during metabolism of absorbed chloridazon in humans and other organisms.

The structure of 5-amino-4-chloro-3-(2H)-pyridazinone, is presented in Figure 1.

Figure 1: Structure of 5-amino-4-chloro-3-(2H)-pyridazinone



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Chloridazon

Chloridazon is a herbicide used pre-plant, pre-emergence and during early post-emergence on sugar beets and red table beets to control certain weeds. Chloridazon is also registered for commercial use on ornamentals including bulb crops and roses (HSDB, 2007).

Chloridazon is currently sold worldwide under several commercial names including: Ashlade CP; Magnum; Pyramin DF; Takron; and Questar. It is generally sold as a formulation that is mixed with water for spray application (Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Chloridazon is moderately soluble in water (422 mg/L) and, in assessing the sorption to soil, a Koc value of 199 L kg-1 has been described suggesting moderate mobility in the environment (Lewis et al., 2007).

Chloridazon is moderately susceptible to degradation in soil under aerobic conditions, with degradation half-lives of 43.1 and 34.7 days under laboratory (20oC) and field conditions respectively; chloridazon is considered to be moderately persistent (Lewis et al., 2007).

2.2.2 Metabolite: 5-Amino-4-chloro-3-(2H)-pyridazinone

5-Amino-4-chloro-3-(2H)-pyridazinone is one of the major metabolites formed through degradation of chloridazon with an estimated maximum occurrence fraction of 0.559 (56%; Lewis et al., 2007).

5-Amino-4-chloro-3-(2H)-pyridazinone has a Koc value of 50 L kg-1 suggesting that the metabolite is mobile in the environment (Lewis et al., 2007).

The metabolite is moderately susceptible to degradation in soil with half-lives of 106.3 and 235.5 days under laboratory (20oC) and field conditions respectively. 5-

Amino-4-chloro-3-(2H)-pyridazinone is considered to be persistent (Lewis et al., 2007).

2.3 Potential routes of human exposure

Occupational exposure to chloridazon may occur through inhalation and dermal contact with this compound at workplaces where it is produced or used. Information on uses suggests that the general population may be exposed to chloridazon via dermal contact during consumer use of products containing chloridazon (HSDB, 2007).

Although no data on routes of exposure of humans to the metabolite 5-amino-4-chloro-3-(2H)-pyridazinone was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of chloridazon, or when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics of parent and metabolite

3.1 Chloridazon

No information is available on the toxicokinetics of chloridazon in humans.
Toxicokinetic studies have shown that chloridazon is readily absorbed from the GI tract of rats, rabbits, cats and dogs. Orally administration of chloridazon to rats (as Pyramin at 625 mg/kg) resulted in maximum tissue levels in the adrenal glands, spleen, heart, kidneys, liver, blood and brain within one to three hours of dosing (HSDB, 2007).

Following oral administration to rats, chloridazon is extensively metabolised and excreted, generally within 24 or 48 hours at low and high doses respectively. The principal excretory route is via urine, with biliary excretion as a minor route. Ten fractions (nine metabolites and one isomer) were detected in urine and 5 fractions in faeces; major urinary metabolites were sulphate and glucuronide conjugates and the major faecal metabolite was a p-hydroxy derivative. The presence of some metabolites was also detected in bile and comprised glucuronide conjugates and p-hydroxy derivatives (USEPA, 2005).

3.2 5-amino-4-chloro-3-(2H)-pyridazinone

No information on the toxicokinetics of 5-amino-4-chloro-3-(2H)-pyridazinone was identified.

4 Toxicity Profile

4.1 Chloridazon

4.1.1 Acute Toxicity

No information is available on the acute toxicity of chloridazon in humans.
Experimentally, chloridazon has low acute toxicity in rats; LD50's are 2140 mg/kg, 2000 mg/kg and 5.4 mg/m3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007).

In animals, chloridazon is not a contact irritant to the skin or eye and no evidence of skin sensitisation has been found (HSDB, 2007). However, repeated dermal exposure has been associated with development of skin rashes in humans (Gosselin et al., 1984).

4.1.2 Repeat dose toxicity

In Wistar (Chbb: THOM (SPF) rats fed chloridazon in the diet at up to 2000 ppm for 25 months, a decrease in body weight was reported in males at 2000 ppm and in female at 1000 ppm. In males given 2000 ppm, increased keratoconjunctivitis,

hyphaemia and smeared fur were evident while females receiving this level showed increased keratoconjunctivitis and poor physical state. In females given 2000 ppm, increased blood cholesterol, calcium and urea were increased and thromboplastin time decreased. A NOEL of 300 ppm (equivalent to 16 mg/kg bw/day) was established (CEPA, 1990).

In Beagle dogs fed a diet containing chloridazon at up to 3600 ppm for 12 months, increased vomiting occurred in high dose males while females given this dose showed reduced food consumption over the first week of treatment. Reduced body weight gains were evident at > 1200 ppm. A NOEL of 400 ppm (equivalent to 11 mg/kg bw/day) was established (CEPA, 1990).

4.1.3 Carcinogenicity and genotoxicity

No information on the carcinogenic or genotoxic potential of chloridazon in humans was identified.

In the dietary studies in rats and dogs described above, no evidence of carcinogenicity was reported.

Experimentally, chloridazon has been evaluated for mutagenicity in both in vitro and in vivo assays. Chloridazon at up to 5000 µg/plate did not show mutagenicity in five strains of S. typhimurium (CEPA, 1990). In a mammalian cytogenic assay at up to 600 mg/kg/day, no evidence of increased micronucleated polychromatic erythrocytes was seen (US EPA, 2005).

In primary rat hepatocytes exposed at up to 1010 µg/ml, no significant increase in net nuclear grain count was seen after 18 hours (CEPA, 1990).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of chloridazon in humans was found.

In a two-generation study, Wistar Chbb: THOM (SPF) rats were fed diets containing chloridazon at up to 1600 ppm. Serum triglycerides levels were reduced in F0 and F1 males and F1 females. Liver weight was increased in F0 high dose females and both F0 and F1 parent animals showed hepatic hydropic swelling and focal fibrosis. A reproductive NOEL of > 1600 ppm was established (CEPA, 1990).

Developmental toxicity was assessed in pregnant Wistar (Chbb:Thom (SPF)) rats given up to 250 mg/kg bw/day by oral gavage on gestation days 6 to15. Maternal toxicity comprised a significant decrease in food consumption and growth, particularly at the highest dose. No treated-related developmental effects were observed; a developmental NOEL of 250 mg/kg was established (CEPA, 1990).

4.2 5-Amino-4-chloro-3-(2H)-pyridazinone

4.2.1 Acute Toxicity

No information on the acute toxic effects of 5-amino-4-chloro-3-(2H)-pyridazinone in humans was identified.

5-Amino-4-chloro-3-(2H)-pyridazinone has low acute toxicity in rats with an oral LD50 of 5000 mg/kg (Lewis et al., 2007).

4.2.2 Repeat dose toxicity

No information on the repeat dose toxicity of 5-amino-4-chloro-3-(2H)-pyridazinone in humans was identified.

Searches were made for published information on the toxicity of 5-amino-4-chloro-3-(2H)-pyridazinone, and by use of the online programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited. Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1: Predicted toxicity data for 5-amino-4-chloro-3-(2H)-pyridazinone using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity/ Mutagenicity	Carcinogenicity	Reproductive & developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$ (range)				
Chloridazon (experimental data)	2140	5.4	NOAEL: 11 mg/kg/day (dogs)	Negative	Negative	Negative
5-amino-4-chloro-3-(2H)-pyridazinone (experimental data)	5000 mg/kg	n/d	n/d	n/d	n/d	n/d
5-amino-4-chloro-3-(2H)-pyridazinone (TOPKAT)	188.5 (33.5-1100)	UE	MTD (feed/drink) 7 mg/kg MTD (oral gavage) UE	Mutagen	Negative	Developmental toxicant
5-amino-4-chloro-3-(2H)-pyridazinone (DEREK)	n/a	n/a	*Plausible* for skin sensitisation in humans	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software; UE –unreliable estimate

Analysis by DEREK identified one structural alert in the molecule and it was concluded that:

it is considered plausible (i.e. there is a weight of evidence) that 5-amino-4-chloro-3-(2H)-pyridazinone will cause skin sensitisation in humans. This endpoint is predicted because 5-amino-4-chloro-3-(2H)-pyridazinone is a hydrazine or precursor.

The analysis by TOPKAT suggested that 5-amino-4-chloro-3-(2H)-pyridazinone was:

non-carcinogenic; but
mutagenic; and

a developmental toxicant.

5 Guidelines and Standards

5.1 Chloridazon

The EC risk classification is: Xi – Irritant: R43; N – Dangerous for the environment: R50, R53. The EC Safety classification is S2, S24, S37, S60, S61. Chloridazon is classified by WHO as 'unlikely to present acute hazard in normal use' and by the US EPA as 'slightly toxic' (Lewis *et al*., 2007).

An ADI of 0.1 mg/kg bw/day has been established for chloridazon from a one year dog feeding study in which a NOEL of 11 mg/kg bw/day was established based on reduced body weight gains; and a safety factor (SF) of 100 was applied (Lewis *et al*., 2007).

5.2 5-amino-4-chloro-3-(2H)-pyridazinone

No ADI has been proposed by an authoritative organisation for 5-amino-4-chloro-3-(2H)-pyridazinone. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

Overall, the toxicity profile for 5-amino-4-chloro-3-(2H)-pyridazinone is not dissimilar to that of the parent, although some suggestion of possible developmental toxicity and mutagenic activity was predicted by one of the predictive systems applied. However, this system also somewhat over-estimated the acute oral toxicity of the compound compared with the experimental data available. The oral MTD predicted by TOPKAT for the metabolite was 7 mg/kg bw (for diet or water administration). Applying a SF of 100 would give a nominal value of 0.07 mg/kg bw; this is close to the ADI of the parent. Given this, it is therefore proposed to adopt a PSDV of 0.1 mg/kg bw/day for 5-amino-4-chloro-3-(2H)-pyridazinone, i.e. the same value as for the parent.

6 References

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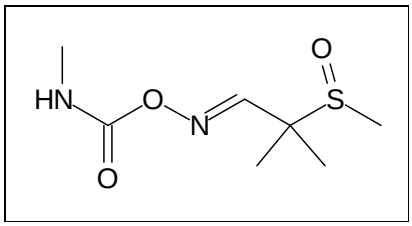
Appendix 12.24 Aldicarb Sulfoxide (i.e. 2-methyl-2-(methylsulfinyl)propanal *o*-((methylamino)carbonyl)oxime)

1 Introduction

Aldicarb sulfoxide (2-methyl-2-(methylsulfinyl)propanal *O*-((methylamino)carbonyl)oxime; CAS No. 1646-87-3) is a major metabolite of the insecticide aldicarb (2-methyl-2-(methylthio)propanal *O*-[(methylamino)carbonyl]oxime; CAS No. 116-06-3). It is formed within plants and surrounding soil and is also a metabolite formed during the metabolism of absorbed aldicarb by humans. Aldicarb sulfoxide also has application, in its own right, as an insecticide and nematicide.

The structure of aldicarb sulfoxide, is presented in Figure 1.

Figure 1: Structure of aldicarb sulfoxide



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Aldicarb

Aldicarb is a systemic insecticide used to control mites, nematodes and aphids on cotton, peanut and soybean crops. Since 1990, it has been restricted to essential use only in the EU and is not used on potatoes due to concerns surrounding groundwater contamination. Aldicarb is currently sold under the commercial names Temik, ENT 27093, OMS 771, and UC 21149. The insecticide is formulated as a granular mix (10-15% a.i.) and applied directly to soil (EXTOXNET, 1996).

2.2 Environmental fate

2.2.1 Parent

Aldicarb has high water solubility (4930 mg/L at 20oC). Studies assessing sorption to soil indicated a Koc value of 30 L kg-1 suggesting mobility in the environment (Lewis et al., 2007).

Degradation studies have shown aldicarb to be moderately persistent in soils with a degradation half-life of 2.4 days under laboratory conditions. The half-life of aldicarb in water has been reported to be between one day and a few months, and this pesticide is considered to be persistent. Degradation rates are considerably faster in surface water for which the half-life is between 5 and 10 days. Aldicarb is also metabolised by plants (EXTOXNET, 1996). Although the degradation of aldicarb in the atmosphere has not been well studied, this is not considered of importance since it is applied beneath the soil in a granular form (IPCS, 1991).

2.2.2 Metabolite: Aldicarb sulfoxide

Aldicarb sulfoxide is one of the major metabolites formed following degradation of aldicarb, with an estimated maximum formation fraction in soil of 0.9 (90%; Lewis et al., 2007).

Aldicarb sulfoxide is highly soluble in water (28000 mg/L at 20oC) and is expected to be very mobile in the environment (Koc value of 1 L kg-1).

Degradation of aldicarb sulfoxide in soil has a half-life of 22 days; the metabolite is not considered to be persistent in the environment (Lewis et al., 2007).

2.3 Potential routes of human exposure

Potential routes of exposure of humans to the parent compound aldicarb may arise through ingestion of contaminated food and water (oral route) or as a result of occupational handling where inhalation or dermal contact may occur (IPCS, 1991).

Exposure of humans to the metabolite aldicarb sulfoxide may similarly occur directly through its use as pesticide or as a result of its ingestion as a food or water contaminated formed from breakdown of aldicarb. Aldicarb sulphoxide may also be formed as a human metabolite from ingested the parent compound (IPCS, 1991).

3 Toxicokinetics

No information is available on the toxicokinetics of aldicarb or aldicarb sulfoxide in humans.

Experimental studies have shown that aldicarb is absorbed almost completely from the GI tract. In one study, Andrawes et al. (1967) administered radio-labelled aldicarb to rats via the oral route and found 80 - 90% recovery of radioactivity in the urine within 24 hr. A further study also reported > 90% recovery of radio-labelled aldicarb in urine after oral administration to rats (Knaak et al., 1966).

In lactating Holstein cows administered radio-labelled aldicarb or aldicarb sulfoxide through oral doses of 0.006 to 0.52 mg/kg/day, 92% of the dose was recovered in urine over a 14 day period (Dorough et al., 1970).

Dermal absorption of aldicarb in an aqueous solution or in toluene has been demonstrated qualitatively in rabbits (Kuhr & Dorough, 1976; Martin & Worthing, 1977) and rats (Gaines, 1969).

In a single dose oral study on female rats administered 0.4 mg/kg 35[S]-aldicarb, showed that aldicarb and its metabolites were widely distributed throughout the body, with tissue showing preferential accumulation (Andrawes et al., 1967). However, some accumulation of aldicarb and its metabolites was noted in the liver of cows after administration of up to 1.2 mg/kg diet of radio-labelled aldicarb in the diet for up to 14 days (Dorough et al., 1970).

In laying hens given a single oral dose (0.7 mg/kg) of an equimolar mixture of aldicarb and aldicarb sulfoxide, accumulation of radiolabel was seen in liver and kidneys for the first 24 hr following administration; levels in fat and muscle were much lower (Hicks et al., 1972).

Metabolic studies in rats have shown the formation of the sulfoxide, sulfone, oxime, sulfoxide, oxime sulfone, nitrile sulfoxide and nitrile sulfone from aldicarb. The sulfoxide and sulfone metabolites have also been shown to be formed in plants (Metcalf et al., 1966; Coppedge et al., 1967). Hydrolysis of aldicarb results in deactivation of its insecticidal activity; however, both the sulfoxide and sulfone metabolites are active anticholinesterase compounds in their own right (Andrawes et al., 1967; Bull et al., 1967; NAS, 1986).

As described previously, experimental studies have shown absorbed aldicarb to be rapidly metabolised and excreted principally in urine (Andrawes et al., 1967; Knaak et al., 1966).

4 Toxicity Profile
4.1 Aldicarb
4.1.1 Acute Toxicity
Acute exposure of workers to aldicarb has been reported to cause symptoms of dizziness, blurred vision, constricted pupils, nausea and abdominal pain; depressed cholinesterase activity has also been reported (NRC, 1983). Very high doses can result in death due to paralysis of the respiratory system. Onset of symptoms occurs between 15 min and 3 hr following exposure and symptoms generally subside within 4 to 12 hr (HSDB, 2005). In rats, aldicarb has high toxicity; LD50's of 0.62 mg/kg, 20 mg/kg and 0.004 mg/m3 have been reported for the oral, dermal and inhalation routes respectively (Lewis et al., 2007).

Aldicarb is not an irritant to the eye or skin (Lewis et al., 2007).

4.1.2 Repeat dose toxicity
A small number of human volunteer studies show that the principal target organ following repeat exposures to aldicarb is the nervous system. In a double-blind placebo-controlled study, 39 men were exposed to aldicarb via their diet at up to 0.075 mg/kg and nine women were given up to 0.05 mg/kg; of the subjects, six women and five men received a dose and a placebo exposure. At all doses, aldicarb treatment resulted in significant inhibition of red blood cell and plasma cholinesterase activity in both males and females. In males, reported clinical signs included sweating, light-headedness, headaches, and salivation and blood pressure changes. Peak effects occurred one hour after administration and the degree and duration of effect were dose-related. In contrast, no clinical signs or symptoms consistent with cholinesterase inhibition were noted in female volunteers. A NOAEL of 0.025 mg/kg bw/day was established based cholinesterase depression (Rhone-Poulenc, 1992; unpublished company report).

A two year feeding study in rats administered aldicarb at up to 0.1 mg/kg bw/day, assessed a number of parameters including, food consumption, mortality (and lifespan), incidence of infection, liver and kidney weight (as percentage of body weight), body weight gain, haematology, incidence of neoplasms, incidence of pathological lesions and brain, and plasma and erythrocyte cholinesterase levels. Animals receiving aldicarb were similar to controls for all parameters, and a NOAEL of ≥ 0.1 mg/kg bw/day was established for systemic toxicity (Union Carbide, 1966; unpublished company report – full study details not available).

4.1.3 Carcinogenicity and mutagenicity
No information on the carcinogenic or mutagenic potential of aldicarb in humans was identified.

Rats administered aldicarb at up to 0.1 mg/kg bw/day in the diet for two years did not show an increased incidence of neoplasms (Union Carbide, 1966; unpublished company report – full study details not available).

In a further study on Greenacres Laboratory (controlled flora) rats administered aldicarb via the diet at up to 0.3 mg/kg bw/day for two years, no increase in incidence of tumours was found. A NOAEL for carcinogenic effects of 0.3 mg/kg bw/day was proposed (Union Carbide, 1972; unpublished company report).

Aldicarb did not show genotoxic activity in bacterial or mammalian gene mutation assays (PSD, 1994).

4.1.4 Reproductive and development toxicity
No information was found on the reproductive and developmental effects of aldicarb in humans.

In a three-generation reproduction study in rats fed aldicarb in the diet at up to 0.7 mg/kg day, histolopathological examination was undertaken on groups of the third generation (F3) animals at weaning and at 90 days of age. No changes in reproductive tissues were seen at any dose tested. However, F2 pups showed decreased body weight at birth at the highest dose tested (0.7 mg/kg/day) and a NOAEL of 0.3 mg/kg day for fetotoxicity was established (Union Carbide, 1974; unpublished company report).

In a developmental toxicity study, pregnant CD rats were administered aldicarb by gavage at up to 0.5 mg/kg bw/day. Decreased maternal body weight and food consumption were reported at 0.25 and 0.5 mg/kg bw/day and some maternal deaths occurred at the highest dose. In offspring, developmental effects were noted at 0.5 mg/kg bw/day; these included significantly increased dilation of lateral ventricles of the brain, poor ossification of the sixth sternebra, together with significantly decreased foetal body weight. At 0.25 and 0.5 mg/kg bw/day, significant increases in the incidence of small haemorrhages on the trunk were also noted. A NOEL for developmental effects of 0.125 mg/kg bw/day was proposed (Rhone-Poulenc, 1988; unpublished company report).

4.2 Aldicarb Sulfoxide
4.2.1 Acute Toxicity
No information on the acute toxicity of aldicarb sulfoxide in humans was identified.
In rats aldicarb sulfoxide is moderately toxic with an oral LD50 of 490 mg/kg (Lewis et al., 2007).

4.2.2 Repeat dose toxicity
No information on the repeat dose toxicity of aldicarb sulfoxide in humans was identified.

Experimentally, aldicarb sulfoxide was fed to rats in their diet at up to 0.6 mg/kg bw/day for 2 years. Other groups received aldicarb sulfoxide in the diet administered as a 1:1 mixture with aldicarb sulfone at up to 1.2 mg/kg bw/day. An elevated mortality rate was noted during the first 30 days of treatment for the high doses groups receiving the sulfoxide alone or the mixture. A slightly higher mortality rate was noted during the later stages in the group given aldicarb sulfoxide alone. A reduced growth performance was also apparent in males fed the highest dose of mixture and a lower plasma cholinesterase activity was noted for the highest dose groups given the mixture or the sulfoxide alone. No treatment-related effects were noted in the lower dose groups and NOELs of 0.3 and 2.4 mg/kg bw/day were proposed for aldicarb sulfoxide and the mixture respectively (IPCS, 1991).

In a further study, aldicarb sulfoxide was administered in the diet to rats at up to 1.0 mg/kg bw/day for up to six months and to Beagle dogs at up to 0.5 mg/kg bw/day, five days per week for three months (Weil & Carpenter, 1968). In rats, plasma and erythrocyte cholinesterase activities were reduced in males and females at the highest dose; males showed the greater sensitivity with the effect also apparent at

doses of 0.25 mg/kg bw/day. A NOAEL in rats of 0.125 mg/kg bw/day was established, based on the depression of cholinesterase activity. In dogs, a slight decrease in body weight gain was noted at the highest dose; no changes in plasma, erythrocyte or brain cholinesterase activities were seen in any dose group. A NOAEL of 0.25 mg/kg bw/day was established based on bodyweight effect.

4.2.3 Carcinogenicity and mutagenicity

No information on the carcinogenic or mutagenic potential of aldicarb sulfoxide in humans was identified.

In the two year rat feeding study described above, no evidence of carcinogenicity was identified (IPCS, 1991).

Aldicarb sulfoxide was shown to be genotoxic using the Ames assay (Godek et al. 1980b; unpublished company report – full study details unavailable).

4.2.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of aldicarb sulfoxide in humans was identified.

Additional information regarding the toxicity of aldicarb sulfoxide was also sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1: Predicted toxicity data for Aldicarb Sulfoxide using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Aldicarb (experimental data)	0.62	0.004	NOAEL 0.01 mg/kg/day Main target: Nervous system	Negative	Negative	Developmental effects (rats)
Aldicarb sulfoxide (experimental data)	490	NR	NOAEL 0.125 mg/kg/day (rats) 0.25 mg/kg/day (dogs)	Positive	Negative	NR
Aldicarb sulfoxide (TOPKAT)	3.8 (0.593-24.6)	3300 (145.3-10,000)	MTD (feed/drink) 0.848 mg/kg MTD (oral gavage) 2.3 mg/kg	UE	UE	Developmental toxicant
Aldicarb sulfoxide (DEREK)	n/a	n/a	*Plausible* for cholinesterase inhibition in humans	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; NR- not reported; MTD – maximum tolerated dose; n/a – prediction not applicable to software

DEREK identified the presence of only one structural alert and the following conclusion was drawn:

It is considered plausible (i.e. there is a weight of evidence) that aldicarb sulfoxide will exhibit cholinesterase inhibition in humans. This endpoint is predicted because aldicarb sulfoxide is an N-Methyl or N,N-dimethyl carbamate compound.

The analysis using TOPKAT suggested that aldicarb sulfoxide was:

a developmental toxicant.

Predictions of the potential carcinogenicity and mutagenicity of aldicarb sulfoxide was not possible using TOPKAT.

5 Guidelines and Standards

5.1 Aldicarb

The EC risk classification is: T+-Very toxic: R26/28; T-Toxic: R24; N – Dangerous for the environment: R50, R53 (Lewis *et al*., 2007). Aldicarb is also classified by WHO as 'extremely hazardous', and by the US EPA as 'highly toxic' (Lewis *et al*., 2007).

An ADI of 0.003 mg/kg bw/day has most recently been established (WHO, 2003) for aldicarb, with a safety factor (SF) of 10, based on cholinesterase depression in a single oral dose study in humans.

5.2 Aldicarb Sulfoxide

No ADI has been proposed by an authoritative organisation for aldicarb sulfoxide. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The lowest experimentally derived NOAEL for aldicarb sulfoxide is 0.125 mg/kg day; this was based on cholinesterase inhibition in a six month feeding study in which rats received aldicarb sulfoxide at up to 1 mg/kg bw/day. Applying a safety factor of 100 would give a PSDV of 0.00125 mg/kg bw/day.

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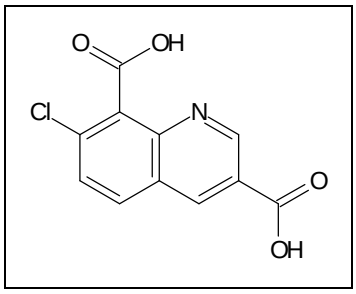
Appendix 12.25 BH518-4 (7-chloro-3,8-quinoline dicarboxylic acid)

1 Introduction

7-chloro-3,8-quinoline dicarboxylic acid (BH518-2) is a major metabolite of the herbicide quinmerac (CAS No. 90717-03-6) in soil. The structure of BH518-2 is presented in Figure 1.

Figure 1: Structure of BH518-2



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of quinmerac

Quinmerac is a new herbicidally active ingredient belonging to the chemical group of quinoline carboxylic acids. Quinmerac, used in formulated mixtures with either chloridazon or metazachlor, offers flexible and potent possibilities for broad spectrum weed control. It is used to control broad-leaved weeds on a range of crops including cereals, rape and beet. Quinmerac is taken up via the root as well as the leaves and is systemic within the plant. The direction of translocation is preferably basipetal. Leaf uptake of quinmerac and its combination partners is enhanced when applied with adjuvants. Quinmerac itself does not influence photosynthesis directly. It enhances, however, the inhibition of photosynthesis by chloridazon synergistically. Quinmerac may stimulate the production of ethylene through its auxin activity in susceptible species. (Lewis et al., 2007; Walter et al, 1994)

It is sold worldwide under the trade names including Oryx, Katamaran and Boomerang.

2.2 Environmental fate

2.2.1 Parent

Quinmerac is highly water soluble (107,000 mg/L at 20oC). In soil, the compound is degraded with a typical half life of 24 days under field conditions. The sorption Koc is measured in the range of 19.2-184.8 mL/g and is sensitive to PH (increases with decreasing pH), suggesting that quinmerac is moderately mobile (Lewis et al., 2007).

2.2.2 Metabolite: BH518-2

BH518-2 is one of the major metabolites formed following degradation or metabolism of quinmerac, with an estimated maximum formation fraction of 0.272 (27%; Lewis et al., 2007).

Information about the environmental fate of BH518-2 is extremely limited. The result from only one study suggested that under field conditions the half life of BH518-2 in soil was 22 days. The sorption Koc is in the range 28-211 mL/g (Lewis et al., 2007).

2.3 Potential routes of human exposure

The potential exposure of humans to the parent quinmerac may occur through ingestion of contaminated food and water (oral route), or through occupational handling or bystander exposure (inhalation or dermal contact).

Although no data on routes of exposure of humans to the metabolite BH518-2was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of quinmerac, or when formed as a human metabolite following ingestion of the parent compound

3 Toxicokinetics
No information is available on the toxicokinetics of quinmerac or BH518-2 in humans.

No experimental information on the toxicokinetics of quinmerac or BH518-2 was identified.

4 Toxicity Profile
4.1 Quinmerac
4.1.1 Acute toxicity
No information is available on the acute toxicity of quinmerac to humans.

In rats, quinmerac showed low toxicity, with LD50 >4070 mg/kg bw and LC50 >5.7 mg/l when given by the oral or inhalation routes. The dermal toxicity of quinmerac was tested in rabbits with LD50 >2000 mg/kg bw. Quinmerac was not irritating to the eye. It may cause sensitization to the skin (BASF, 2009).

4.1.2 Repeat dose toxicity
No information on the repeat dose toxicity of quinmerac in humans, or experimental data was identified

4.2 BH518-2
No information on the repeat dose toxicity of BH518-2 in humans was identified.

Searches were made for publicly available information on the toxicity of BH518-2 and by use of the online programme ChemIDPlus (http://chem.sis.nlm.nih.gov/chemidplus/chemidlite.jsp). However, the toxicity information identified was extremely limited.

Additional information was, therefore, sought through application of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Summaries of the toxicity data predicted for BH518-2 from these tools are summarised below (Table 1).

DEREK did not identify the presence any structural alerts in the BH518-2 molecule.

The analysis by TOPKAT suggested that BH518-2 was:

- non-carcinogenic
- non-mutagenic
- not a developmental toxicant

Table 1 Predicted toxicity data for BH518-2 using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Quinmerac (experimental data)	>5000	>5.7	n/d	n/d	n/d	n/d
BH518-2 (TOPKAT)	3000 (532.1-10,000)	10,000 (5400-10,000)	MTD (feed/drink) 5.4 mg/kg MTD (oral gavage) UE	Negative	Negative	Negative
BH518-2 (DEREK)	n/a	n/a	No alert	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software; UE – unreliable estimate; not derived

5 Guidelines and Standards

5.1 Quinmerac

EC risk classification for quinmerac is; N - Dangerous for the environment: R50, R53. EC safety classification is S60, S61. Quinmerac is classified by WHO as 'unlikely to present acute hazard in normal use'.

An ADI of 0.079 mg/kg bw/day has been established for quinmerac (Lewis *et al*., 2007). This was based on a dog study with safety factor of 100, however the reference of the study could not be identified.

5.2 BH518-2

Due to the lack of experimental data, no ADI has been proposed for BH518-2 by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The highest oral MTD predicted by TOPKAT for BH518-2 is 5.4 mg/kg bw/d (feed and water administration). Applying a SF of 100 would give a nominal value of 0.054 mg/kg bw, which is very similar to the established ADI of 0.079 mg/kg bw/d for the parent quinmerac. Given this, it is therefore proposed to adopt a PSDV of 0.079 mg/kg bw/d for BH518-2, i.e. the same value as for the parent.

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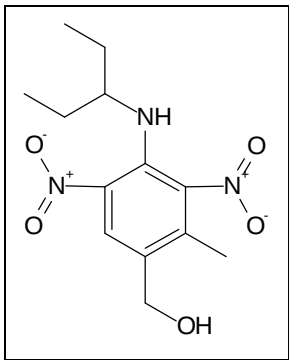
Appendix 12.26 4-[(1-Ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol

1 Introduction

4-[(1-Eethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol is a major metabolite of the herbicide pendimethalin (*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine; CAS No. 40487-42-1).

The structure of 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol is presented in Figure 1.

Figure 1: Structure of 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Pendimethalin

Pendimethalin is a selective herbicide used pre- and post-emergence to control annual grasses and annual broad-leaved weeds in field corn, potatoes, rice, cotton, soybeans, tobacco, peanuts and sunflowers (HSDB, 2003). Pendimethalin is sold worldwide under the trade names, Blazer, Bunker, Claymore, PDM 330 EC, PicoMax, Picona, PicoPro, Stomp, PicoStomp, Pendimethalin 330 EC and Trump (Lewis et al., 2007).It is often supplied as an emulsifiable or emulsion concentrate that is mixed with water and used as a spray (Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Pendimethalin has low solubility in water (0.33 mg/L at 20oC). In assessing the sorption to soil, a Koc value of 15744 l/ kg has been reported suggesting that it is not mobile in the environment (Lewis et al., 2007).

Studies have shown pendimethalin is moderately degraded in soils with half-lives of 123 and 90 days, under laboratory (20oC) and field conditions respectively. The compound is moderately persistent in the environment (Lewis et al., 2007).

During use, pendimethalin will be released into the atmosphere where it will be present in the vapour phase where it can react with photochemically-produced hydroxyl radicals; the estimated atmospheric half-life of 12.7 hr (HSDB, 2003).

2.2.2 Metabolite: 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol

4-[(1-Ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol is one of the metabolites formed following degradation or, to a limited extent, metabolism of pendimethalin.

No information on the physicochemical properties or environmental fate of 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol was identified.

2.3 Potential routes of human exposure

Exposure of humans to pendimethalin will primarily be through occupational dermal contact and inhalation and ingestion of aerosols during mixing and application and by contact with treated plants and soil. The general population is most likely to be exposed to pendimethalin through ingestion of and dermal contact with contaminated water, including rain water. This is most likely to occur near agricultural areas during the growing season (HSDB, 2003).

Although no data on routes of exposure of humans to the metabolite 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of pendimethalin, and, to a limited extent, when formed as a human metabolite following ingestion of the parent compound

3 Toxicokinetics

No information is available on the toxicokinetics of pendimethalin in humans.

Experimentally, the toxicokinetics of pendimethalin was established in a single oral dose study in which rats were given 7.3 and 37 mg/kg 14[C]-pendimethalin. Absorption from the GI tract was shown to be very low and a large proportion of pendimethalin was excreted unchanged in the faeces. Rapid metabolism of absorbed pendimethalin occurred in the kidneys and liver, with metabolites excreted in urine. Following a period of 24 hr after administration of a 37 mg/kg dose, over 90% was seen to be recovered in faeces and urine, with 96% recovery after 4 days (Zulalian, 1990).

Tissue distribution of absorbed pendimethalin was found to be minimal with 0.3 ppm being recovered in all tissues and 0.9 ppm in fat 4 days following administration (Zulalian, 1990).

Metabolism of pendimethalin was shown to involve hydroxylation of the 4-methyl and the N-1-ethyl group, oxidation of these alkyl groups to carboxylic acids, nitro reduction, cyclization, and conjugation (Zulalian, 1990).

No information on the toxicokinetics of 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol was identified.

4 Toxicity Profile

4.1 Pendimethalin

4.1.1 Acute Toxicity

No information is available on the acute toxicity of pendimethalin in humans.

In rats, pendimethalin has moderate acute toxicity; LD50's are 3189 mg/kg, 2000 mg/kg and 320 mg/m3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007).

Pendimethalin is not a skin irritant or sensitizer in rabbits or guinea pigs, but causes mild eye irritation in rabbits. Inhalation of dusts or fumes may be mildly to moderately irritating to the linings of the mouth, nose, throat, and lungs (EXTOXNET, 1996).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of pendimethalin in humans was identified.

Experimentally, the principal target organs of repeated exposure for pendimethalin are the liver and, in rats only, the thyroid (EC, 2003).

In Beagle dogs fed for two years on diets containing pendimethalin at up to 200 mg/kg bw/day, serum alkaline phosphatise (SAP) activity was increased at the two highest doses. Increased liver weight and associated inflammatory changes and haemosiderosis were also noted in the two highest dose groups. A NOEL of 12.5 mg/kg/day was established, based on the hepatic toxicity (IRIS, 1991).

In rats fed pendimethalin in the diet for 56 days for study of thyroid function only, exposure to 500 ppm (31 mg/kg bw/day) caused changes in serum chemistry by day 28 ; these comprised statistically significant decreases (38%) in total serum T4, reverse T3 (25%) and total T4 (28%) and an increase in free T3 (13%).

4.1.3 Carcinogenicity and mutagenicity

No information on the carcinogenic and mutagenic potential of pendimethalin in humans was identified.

In the 56 day rat thyroid function study reported above, histopathological changes included increased thyroid follicular cell height (40%) and decreased colloid areas (37%); some of these changes were detectable by day 3 of treatment. Although such changes may be indicative of carcinogenic potential, no tumour diagnosis was apparent in the study. However, a NOEL of 100 ppm (10 mg/kg/day) was established for potential carcinogenic effects (CEPA, 1999) and pendimethalin has been classified by the US EPA as a Group C, possible human carcinogen (US EPA, 1997).

In mice fed a diet containing 75 mg/kg bw/day of pendimethalin for 18 months, no evidence of increased tumour formation was reported (EXTOXNET, 1996).

Experimentally, pendimethalin was negative in a number of in vitro and in vivo mutagenicity assays including tests on live animals and mammalian and bacterial cell cultures (EXTOXNET, 1996).

4.1.4 Reproductive and development toxicity

No information was identified on the reproductive or developmental effects of pendimethalin on humans.

Experimentally, in a three-generation rat study in which pendimethalin was administered via the diet at up to 250 mg/kg/day, reduced offspring numbers at birth and offspring growth (from weaning to maturity) were noted at the mid and high doses. No effects were observed below 30 mg/kg/day, and a reproductive NOEL of 25 mg/kg/day was established (IRIS, 1991).

Teratology studies in pregnant rats administered pendimethalin in the diet at up to 500 mg/kg bw/day have been reported. No fetotoxic or teratogenic effects were noted and a NOAEL of 500 mg/kg bw/day was established (IRIS, 1991).

4.2 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol

No information on the repeat dose toxicity of 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol in humans was identified.

Searches were made for publicly available information on the toxicity of 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol, and by use of the online

programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.
Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Analysis by DEREK identified the presence of a number of structural alerts in the molecule, and the following conclusions were drawn:

- It is considered plausible (i.e. there is a weight of evidence) that 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol will exhibit carcinogenicity in humans. This endpoint is predicted because 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol is an aromatic nitro compound;

- It is considered plausible (i.e. there is a weight of evidence) that 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol will exhibit hepatotoxicty in humans. This endpoint is predicted because 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol is an aromatic nitro compound; and

- It is considered OPEN (i.e. there is no overall weight of evidence) that 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol will exhibit mutagenicity in humans. This endpoint is predicted because 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol is an aromatic nitro compound.

The analysis by TOPKAT suggested that 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol was:

- non-carcinogenic;
- non-mutagenic; and
- not a developmental toxicant.

Table 1: Predicted toxicity data for 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Pendimethalin (experimental data)	3189	320	NOAEL: 12.5 mg/kg/day Main target organs: Liver and thyroid (rats)	Negative	Some evidence in rats (thyroid).	Negative
4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol (TOPKAT)	1100 (284.8-4600)	10,000 (1200-10,000)	MTD (feed/drink) 189.7 mg/kg MTD (oral gavage) 189.7 mg/kg	Negative	Negative	Negative

4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol (DEREK)	n/a	n/a	*Plausible* for hepatotoxicity in humans	*OPEN* in humans	*Plausible* in humans	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software; UE- unreliable estimate

5 Guidelines and Standards

5.1 Pendimethalin

The EC risk classification is: Xi - Irritant: R43; N - Dangerous for the environment: R50, R53 and EU safety category is S2, S22, S29, S37, S60, S61. Pendimethalin is classified by WHO as 'slightly hazardous' and the US EPA as 'slightly toxic' (Lewis *et al.,* 2007).

An ADI of 0.125 mg/kg bw/day was established for pendimethalin (EU, 2003) using a safety factor (SF) of 100, based on the two feeding study in Beagle dogs administered pendimethalin at up to 200 mg/kg bw/day (Lewis *et al*., 2007).

5.2 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol

Due to the lack of availability of experimental data, it has not been possible to establish a robust NOEL or NOAEL for 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol, and no ADI has been proposed by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The general repeat dose toxicity profile for 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol is overall predicted to be somewhat less than that of the parent although the acute oral (but not inhalation) toxicity is predicted to be similar or possibly slightly greater than that of the parent. The highest oral MTD predicted by TOPKAT for 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol is 189.73 mg/kg bw (irrespective of method of oral administration). Applying a SF of 100 would give a nominal value of 1.89 mg/kg bw, which would be considerably higher than the established ADI of 0.125 mg/kg/bw for the parent pendimethalin. However, given the predicted hepatotoxicity and the concerns regarding potential mutagenic and carcinogenic potential by one of the two predictive systems utilised, it is therefore proposed to adopt a PSDV of 0.125 mg/kg bw/d for 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol (i.e. the same value as for the parent); this will provide an overall SF of greater than 1500 .

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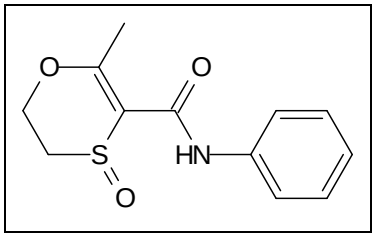
Appendix 12.27 Carboxin sulfoxide (1,4-oxathin-3-carboxamide, 5,6-dihydro-2-methyl-*N*-phenyl-4-oxide)

1 Introduction

Carboxin sulfoxide (1,4-oxathin-3-carboxamide, 5,6-dihydro-2-methyl-*N*-phenyl-4-oxide; CAS No. 17757-70-9) is a major metabolite of the fungicide carboxin (5,6-dihydro-2-methyl-*N*-phenyl-1,4-oxathiin-3-carboxamide; CAS No. 5234-68-4) formed within surrounding soil and during metabolism of absorbed carboxin in humans.

Carboxin sulfoxide has also been used as a fungicide in its own right. The structure of carboxin sulfoxide is presented in Figure 1.

Figure 1: Structure of Carboxin sulfoxide



2 Use, environmental fate and potential human exposure routes

2.1 Uses of Carboxin and Carboxin Sulfoxide

2.1.1 Carboxin

Carboxin is a fungicide used for seed treatment to control smuts and bunts (particularly loose smut, Ustilago spp.) on barley, wheat, and oats and for seedling diseases (particularly Rhizoctonia spp.) of barley, wheat, oats, rice, cotton, peanuts, soya beans, vegetables, maize, sorghum and other crops (HSDB, 2005).

Carboxin is currently sold worldwide under the commercial name Vitavax 200FF. It is generally sold as a flowable concentrate that is diluted with water for use as a seed treatment (Lewis et al., 2007).

2.1.2 Carboxin Sulfoxide

Carboxin sulfoxide is a fungicide used as a foliar spray for control of rust diseases of ornamentals grown in enclosed commercial structures (such as greenhouses, shadehouses and interior-scapes). Carboxin sulfoxide was first registered as an active ingredient in the US in 1971. Due to low sales and the limited disease spectrum it has not been marketed in the US since 2003, however it is still approved for use in many European countries and Australia (HSDB, 2005; Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Carboxin is moderately soluble in water (130 mg/L) and, in assessing the sorption to soil, a Koc value of 99 L kg-1 has been described suggesting moderate mobility in the environment (Lewis et al., 2007).

Carboxin is extremely susceptible to degradation in soil under aerobic conditions, with degradation half-lives of 0.58 and 3.3 days under laboratory (20oC) and field conditions respectively. Carboxin is not considered to be persistent (Lewis et al., 2007).

Carboxin sulfoxide is the major metabolite formed through degradation of carboxin with an estimated maximum occurrence fraction of 0.780 (78%; Lewis et al., 2007).

2.2.2 Metabolite: Carboxin Sulfoxide

Carboxin sulfoxide is highly soluble in water (587.7 mg/L) and, in assessing the sorption to soil, a Koc value of 96 L kg-1 has been described suggesting moderate mobility in the environment (Lewis et al., 2007).

The metabolite is moderately susceptible to degradation in soil, with half-lives of 39.5 and 59.5 days under laboratory (20oC) and field conditions respectively; it is considered moderately persistent (Lewis et al., 2007).

2.3 Potential routes of human exposure

Occupational exposure to carboxin may occur through inhalation and dermal contact with this compound at workplaces where it is produced or used. The general population may be exposed through ingestion of food and water contaminated with carboxin, (HSDB, 2005).

Although no data on routes of exposure of humans to the metabolite carboxin sulfoxide was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of carboxin, and, to a limited extent, when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of carboxin in humans.

In Sprague-Dawley rats orally dosed with 14[C]-carboxin at 5 or 150 mg/kg bw, total recovery of radiolabel ranged from 77 – 82%, with excretion occurring primarily via urine (65 – 77%); faecal excretion accounted for only 6 – 11%. Rates of excretion were higher at the low dose suggesting saturation of absorption, metabolism or excretory mechanisms may occur. After absorption, carboxin is widely distributed, with tissue residues accounting for 0.26 – 0.40% of administered dose after 72 hours. The highest levels of carboxin and its metabolites were in liver (0.21%), red blood cells and kidney; lowest levels were in adipose tissue, brain, gonads, bone and skeletal muscle. Carboxin was extensively metabolised with six urinary metabolites being indentified. It appears that the metabolic pathway includes oxidation of both oxathiin and aromatic rings, hydrolysis of amide bonds and conjugation of the aniline and phenol moieties. The predominant metabolite was parahydroxylated carboxin sulfoxide (Markham, 1992).

No information on the toxicokinetics of carboxin sulfoxide was identified.

4 Toxicity Profile

4.1 Carboxin

4.1.1 Acute Toxicity

No information on the health effects following acute exposure in humans to carboxin was found.

Experimentally, carboxin has low acute toxicity in rats; LD50's are 2588 mg/kg, 4000 mg/kg and 4.7 mg/m3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007).

In animals, carboxin is not a contact irritant to the skin or eye but is classified as a skin sensitiser based on positive results in Magnusson and Kligman tests (EU, 2006).

4.1.2 Repeat dose toxicity
No information relating to adverse effects in humans following repeated exposure to carboxin was found.

In male and female Crl:CD BR rats fed carboxin in the diet at up to 400 ppm (males) and 600 ppm (females) for two years, survival was significantly lower in males at 400 ppm (during weeks 65-102) and in females at 600 ppm (during weeks 85-102). Other effects included reduced body weight gain and increased water consumption in males given ≥ 200ppm and in females at 600 ppm. In animals given ≥ 200 ppm changes in clinical chemistry and urine parameters (calcium creatinine, phosphorous and urea nitrogen levels) were apparent. Necroscopy showed diffuse light and rough surfaces and cystic kidneys in both sexes and a slight increase in liver weight mass in males. Histopathological examination revealed chronic nephritis, tubular mineralisation, tubular cell degeneration, tubular epithelium hyperplasia and cysts in the kidneys of both sexes at the highest dose. Increased incidence of parathyroid hyperplasia and fibrous osteodystrophy of the femur were noted in all treated male groups and in mid and high dose females. In addition, the incidence of ovarian cysts was increased at all treated group. A NOAEL of 20 ppm (equivalent to 1.05 mg/kg bw/day) was established based on the kidney changes in both sexes (Kehoe, 1991).

4.1.3 Carcinogenicity and genotoxicity
No information on the carcinogenic or genotoxic potential of carboxin in humans was identified.

In the two year dietary studies in rats described above, an increased incidence of hepatic carcinoma was noted in males at the high dose of 400 ppm. A NOAEL of 200 ppm (equivalent to 8.65 mg/kg bw/day) was established for carcinogenicity in males and a value of > 600 ppm (33.48 mg/kg bw/day) in females (Kehoe, 1991).

The genotoxicty of carboxin has been assessed in an Ames assay, a chromosome aberration test and a Unscheduled DNA synthesis (UDS) assay. Gene mutation assays were negative in both bacterial and mammalian cells (Brusick, 1982). However, carboxin was clastogenic in the presence of metabolic activation (Galloway, 1982) and positive in the unscheduled DNA repair test in rat hepatocytes (Myhr, 1982).

4.1.4 Reproductive and development toxicity
No information on the reproductive and developmental toxicity of carboxin in humans was identified.

In a two-generation reproductive toxicity study, Crl:CD Br rats were fed carboxin in the diet at up to 400 ppm in males and 600 ppm in females for 10 weeks prior to mating and though to weaning. Parental toxicity comprised reduced body weight gain associated with reduced food consumption at 400 ppm in males and 600 ppm in females and increased kidney lesions at ≥ 200 ppm in males and ≥ 300 ppm in females. No reproductive effects were noted. Reproductive NOAELs of 400 ppm (equivalent to 20 mg/kg bw/day) and 600 ppm (30 mg/kg bw/day) were established for males and females respectively (Kehoe, 1991b). The mean body weight of F2 pups were consistently below that of controls. Hence a developmental NOAEL of 5 ppm (90 mg/kg bw/day) was established (Schardein, 1989).

4.2 Carboxin sulfoxide
4.2.1 Acute Toxicity

No information on the acute toxic effects of carboxin sulfoxide in humans was identified.

Experimentally, carboxin sulfoxide has moderate acute toxicity in rats; the oral LD50 is 2000 mg/kg (Lewis et al., 2007).

4.2.2 Repeat dose toxicity

No information on the repeat dose toxicity of carboxin sulfoxide in humans was identified.

Searches were made for other published information on the toxicity of carboxin sulfoxide, including use of the online programme ChemIDPlus (US NLM, 2003). However, no further relevant data were identified.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1: Predicted toxicity data for carboxin sulfoxide using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$ (range)				
Carboxin (experimental data)	2588	4.7	NOAEL: 1.05 mg/kg/day; Target organ - Liver	Genotoxic	Hepatocellular carcinoma (rats)	Decreased body weight F2 pups
Carboxin Sulfoxide (experimental data)	2000	n/d	n/d	n/d	n/d	n/d
Carboxin Sulfoxide (TOPKAT)	3000 (889 - 9900)	23.8 (2.2 – 255.7)	MTD (feed/drink) 2000 mg/kg MTD (oral gavage) 2000	Non-mutagen	Negative	Negative
Carboxin Sulfoxide (DEREK)	n/a	n/a	*Plausible* for methaemogl -obinaemia in humans	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software

DEREK identified the presence of one structural alert and the following conclusion was drawn:

it is considered plausible (i.e. there is a weight of evidence) that carboxin sulfoxide will cause methaemoglobinaemia in humans.This endpoint is predicted because carboxin sulfoxide is a simple aniline or precursor.

The analysis by TOPKAT suggested that carboxin sulfoxide was:

non-mutagenic;
non-carcinogenic; and
not a developmental toxicant.

5 Guidelines and Standards

5.1 Carboxin

The EC risk classification is: Carcinogen category 3: R40; Xi - Irritant: R43; N - Dangerous for the environment: R50, R53. The EC safety classification is S36/37, S60, S61. Carboxin is classified by WHO as 'unlikely to present acute hazard in normal use' and by the US EPA as 'slightly toxic' (Lewis *et al*., 2007).

An ADI of 0.0016 mg/kg bw/day has been established for carboxin using a safety factor (SF) of 100, based on a two-year rat dietary study in which a NOAEL of 1.05 mg/kg bw/day was established based on kidney changes (Lewis *et al*., 2007).

5.2 Carboxin Sulfoxide

No ADI has been proposed by any authoritative organisation for carboxin sulfoxide. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

Overall, the predicted toxicity profile for carboxin sulfoxide suggests that it is less toxic than the parent. The oral MTD predicted by TOPKAT is 2000 mg/kg bw/day, compared with a NOAEL of only 1.05 mg/kg bw/day for the parent. Applying a SF of 100 to the MTD would give a nominal value of 20 mg/kg bw/day which is markedly higher than the parental compound's ADI (0.0016 mg/kg bw/day) for the parent carboxin. Given this, it is – on a highly precautionary basis – therefore proposed to adopt a PSDV of 0.0016 mg/kg bw/d for carboxin sulfoxide, i.e. the same value as for the parent; this would give an overall SF >10,000.

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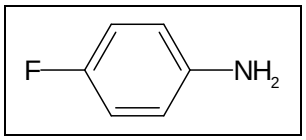
Appendix 12.28 4-Fluoroaniline

1 Introduction

4-fluroaniline is a metabolite of the herbicide picolinafen (*N*-(4-fluorophenyl)-6-[3-(trifluoromethyl)phenoxy]-2-pyridinecarboxamide; CAS No. 137641-05-5) and is formed within the surrounding soil following application of the parent to plants. It is also formed through metabolism of absorbed picolinafen in humans.

The structure of 4-fluoroaniline, is presented in Figure 1.

Figure 1: Structure of 4-fluroaniline



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Picolinafen

Picolinafen is a herbicide that is used for post-emergence application in winter cereals such as wheat, barley, oats, triticale and cereal rye, for control or suppression of broadleaf weeds (APVMA, 2000).

Picolinafen is currently sold worldwide under several commercial names including Flight, Orient, PicoMax, Picona, PicoPro, PicoStomp. It is generally supplied as wettable granules that are mixed with water for spray application (Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Picolinafen has low solubility in water (0.047 mg/L) and, in assessing the sorption to soil, a Koc value of 23400 L/kg has been described suggesting no mobility in the environment (Lewis et al., 2007).

Picolinafen is moderately susceptible to degradation in soil under aerobic conditions with degradation half-lives of 7.5 and 30 days under laboratory (20oC) and field conditions respectively. picolinafen is considered to be moderately persistent (Lewis et al., 2007).

2.2.2 Metabolite: 4-fluoroaniline

4-fluoroaniline is one of the metabolites formed through metabolism or environmental degradation of picolinafen.

No information relating to either the physiochemical properties or environmental fate of the 4-fluoroaniline was found.

2.3 Potential routes of human exposure

Occupational exposure to picolinafen may occur through inhalation and dermal contact at workplaces where it is produced or used. The general population may be exposed to picolinafen via dermal contact (APVMA, 2000).

Although no data on routes of exposure of humans to the metabolite 4-fluoroaniline was identified, it is plausible that exposure may occur as a result of its ingestion in

water contaminated from the breakdown of picolinafen, and, to a limited extent, when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics of parent and metabolite
No information is available on the toxicokinetics of picofinalin in humans.

Toxicokinetic studies have shown that radiolabelled picolinafen is readily absorbed (up to 60%) from the GI tract of rats. Although widely distributed following absorption, less than 0.5% of radioactivity was recovered in tissues after seven days, suggesting a very low bioaccumulation potential. Picolinafen is metabolised by cleavage of the amide bond followed by a variety of biotranformations (including N-acetylation, hydroxylation, methylation, dehalogenation and the formation of glucuronic, mercapturic and sulfate conjugates). Distribution of metabolites labelled on the pyridine ring was predominantly to fat, liver and kidneys while metabolites labelled on the pyridine ring (the predominant metabolites) localised to the blood, liver, spleen and lungs. Unabsorbed and unmetabolised parent compound accounted for 95% of the radioactivity found in faeces and insignificant amounts of radiolabelled picolinafen metabolites were found in exhaled air (<0.1% of administered dose). Biliary excretion was the major route of excretion of metabolites of pyridine-ring labelled Picolinafen while urinary excretion was the major route for aniline-ring metabolites (APVMA, 2000; EU, 2002).

No information on the toxicokinetics of 4-fluoroaniline was identified.

4 Toxicity Profile
4.1 Picolinafen
4.1.1 Acute Toxicity
No information on the acute effects of picolinafen in humans was found.
Experimentally, picolinafen has low acute toxicity in rats; LD50's are 5000 mg/kg, 4000 mg/kg and 5.9 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007).

In animals, picolinafen is irritant to eyes but not skin. No evidence of skin sensitisation has been shown (Lewis et al., 2007).

4.1.2 Repeat dose toxicity
No information on the adverse health effects following repeated exposure of humans to Picolinafen was found.

Experimentally, in rats fed diet containing picolinafen at up to 500 ppm for two years, haemoglobin, haematocrit and red blood cell count were reduced at 3 and 6 months at 250 and 500 ppm. Spleen weights were increased at 500 ppm and the amount of brown pigment in the spleen was increased at 250 and 500 ppm at both 12 and 24 months. A NOEL of 50 ppm was established (equivalent to 2.4 and 3.0 mg/kg bw/day for males and females respectively; APVMA, 2000; EU, 2002).

Dogs fed diet containing picolinafen at up to 1500 ppm for 12 months showed reduced body weight gain in all treated male groups. Haemoglobin, haematocrit and red blood cells were transiently reduced while platelet counts were increased throughout the study period in females given 1500 ppm . A transient increase in platelet count was also seen at 150 ppm. In males given 1500 ppm, mean corpuscular volume was increased throughout treatment and enlarged thyroids and increased thyroid weights were observed. Histopathologically, in both males and females, thyroid follicular hypertrophy was observed at 1500 ppm and hyperplasia in

females only. A NOEL could not be established as body weight gains were reduced at all doses; a LOEL of 50 ppm was established (equivalent to 1.4 and 1.6 mg/kg bw/day in males and females respectively; APVMA, 2000; EU, 2002).

4.1.3 Carcinogenicity and genotoxicity

No information on the carcinogenic or genotoxic effects following repeated exposure of humans to picolinafen was found.

In the two year rat feeding study described above, no effect on neoplasms was seen; picolinafen is not considered to be carcinogenic (APVMA, 2000; EU, 2002).

No genotoxic potential has been demonstrated for picolinafen using bacterial and mammalian mutation assays in vitro. Furthermore, the compound did not induce formation of micronuclei in mouse bone marrow cells in vivo nor did it increase the frequency of chromosomal aberrations in mammalian cells in vitro (APVMA, 2000).

4.1.4 Reproductive and development toxicity

No information was found on the reproductive and developmental effects of picolinafen in humans.

In a two-generation study in rats fed diet containing picolinafen at up to 500 ppm, food consumption was slightly increased in F0 rats at 250 and 500 ppm and in F1 parental rats at 500 ppm prior to mating. Across all generations, haematological effects were reported as reduced haemoglobin, haematocrit and red blood cell count and increased mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin volume and reticulocyte and leukocyte counts. These changes were generally observed at 500 ppm with some parameters affected at 250 ppm. In adult males, liver and spleen weights were increased at 500 ppm and in adult females spleen weights were increased at 250 and 500 ppm. Histopathological examination of the spleens showed congestion, increased amounts of brown pigment and extramedullary haematopoiesis at 250 and 500 ppm. In neonates, the only finding was haematological changes at 250 and 500 ppm. Both fertility and reproduction parameters were unaffected at any dose. A reproductive NOEL of 50 ppm was established, equivalent to 3.7 and 2.4 mg/kg bw/day for males and females respectively (APVMA, 2000; EU, 2002).

Developmental toxicity of picolinafen was assessed in pregnant rats administered up to 1000 mg/kg bw/day by oral gavage on days 6 to 9 of gestation. Maternal toxicity was noted with a reduction in body weight associated with reduced food consumption at ≥ 500 ppm. Spleen weights were increased in dams at 100 mg/kg bw/day and bodyweight-relative liver weights were slightly increased at 500 mg/kg bw/day. In all treatment groups, haemoglobin, haematocrit and red blood cells were reduced and mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin volume, nucleated red blood cell and reticulocyte counts increased. The incidence and severity of extramedullary haematopoiesis and haemosiderosis in the spleen was increased in a dose-dependent manner. Developmental effects included increased incidence of bipartite ossification in the thoracic vertebrae in foetuses at 1000 mg/kg bw/day but no other developmental parameters were altered. The NOEL for foetal toxicity was established as 500 mg/kg bw/day (APVMA, 2000; EU, 2002).

In pregnant rabbits, oral gavage at up to 50 mg/kg bw/day on days 6-28 of gestation resulted in maternal toxic effects including soft or liquid faeces at 50 mg/kg bw/day and reduced body weight gain and food consumption at 20 or 50 mg/kg bw/day. In addition, haemoglobin, haematocrit and red blood cells were reduced and mean

corpuscular volumes and reticulocyte counts increased in the groups given 20 or 50 mg/kg bw/day. Congestion and haemosiderosis in the spleen were increased in incidence and severity at these doses while increased extramedullary haematopoiesis was apparent at 50 mg/kg bw/day. Of the developmental parameters, numbers of resorptions and incidences of fused sternal centra in fetuses were increased at 50 mg/kg bw/day. A NOEL for embryotoxicity and fetotoxicity was established as 20 mg/kg bw/day (APVMA, 2000; EU, 2002).

4.2 4-Fluoroaniline

4.2.1 Acute Toxicity

No information on the effects of acute exposure of humans or animals to 4-fluoroaniline was found.

4.2.2 Repeat dose toxicity

No information was found on the effects following repeated exposure to 4-fluoroaniline in humans.

Searches were made for published information on the toxicity of 4-fluoroaniline, including use of the online programme ChemIDPlus (US NLM, 2003). However, no relevant information was identified.

Additional information was, therefore, sought through use of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1: Predicted toxicity data for 4-fluoroaniline using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Picolinafen (experimental data)	5000	5.9	NOAEL: 1.4 mg/kg/day (dogs) Target organ: red cells, spleen, liver (also thyroid in dogs)	Negative	Negative	Increased resorption rate; decreased foetal body weights (rabbit)
4-Fluoroaniline (TOPKAT)	480.9 (128.5-1800)	7100 (873.3-10,000)	MTD (feed/drink) 29.4 mg/kg MTD (oral gavage) 29.4 mg/kg	Mutagen	Positive	Negative
4-fluoroaniline (DEREK)	n/a	n/a	*Plausible* for methaemogl-obinaemia and skin sensitisation in humans	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software;

DEREK concluded that the presence of two structural alerts and the following conclusions were drawn:

it is considered plausible (i.e. there is a weight of evidence) that 4-fluoroaniline will cause methaemoglobinaemia in humans. This endpoint is predicted because 4-fluoroaniline is a simple aniline or precursor.

it is considered plausible (i.e. there is a weight of evidence) that 4-fluoroaniline will cause skin sensitisation in humans. This endpoint is predicted because 4-fluoroaniline is an aromatic primary or secondary amine. .

The analysis by TOPKAT suggested that 5-amino-4-chloro-3-(2H)-pyridazinone was:

carcinogenic; and
mutagenic; but
not a developmental toxicant

5 Guidelines and Standards

5.1 Picolinafen

Picolinafen is not classified under the EU risk or safety classifications, or by WHO or the US EPA (Lewis *et al.*, 2007).

An ADI of 0.014 mg/kg bw/d has been established for picolinafen using a safety factor (SF) of 100, based on a NOAEL of 1.4 mg/kg bw/day established in based a 1 year dog feeding study (Lewis *et al*., 2007).

5.2 4-fluoroaniline

No ADI has been proposed by any authoritative organisation for 4-fluoroaniline. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

According to the predicted toxicity profile for 4-fluoroaniline using TOPKAT, the metabolite may possess mutagenic and carcinogenic activities not found for the parent, and is predicted to have greater acute toxicity. However, similar alerts were not produced by DEREK and the oral MTD predicted by TOPKAT for 4-fluoroaniline was 29.4 mg/kg bw/day (irrespective of form of oral administration). Applying a SF of 100 would give a nominal value of 0.29 mg/kg bw/day which would be significantly higher than the established ADI of 0.014 mg/kg bw/day for the parent picolinafen. Given this, it is considered adequately precautionary to adopt a PSDV of 0.014 mg/kg bw/d 4-fluoroaniline, i.e. the same value as for the parent. This would give an overall SF of above 2000.

6 References

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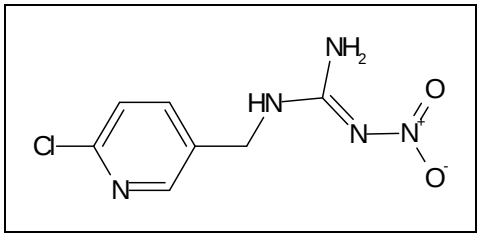
Appendix 11.29 1-(6-Chloro-pyridine-3-methyl)-N-nitroguanidine

1 Introduction

1-(6-Chloro-pyridine-3-methyl)-N-nitro guanidine is a metabolite of the insecticide imidacloprid (5-amino-4-chloro-2-phenyl-3(2*H*)-pyridazinone; CAS No. 138261-41-3) and is formed within the surrounding soil following administration of the parent to plants. The metabolite is also formed during metabolism of absorbed imidacloprid in humans.

The structure of 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine, is presented in Figure 1.

Figure 1: 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Imidacloprid

Imidacloprid is an insecticide used on soil, seed and foliage for the control of sucking insects including rice hoppers, aphids, thrips, whiteflies, termites, turf insects, soil insects and some beetles. It is most commonly used on rice, cereal, maize, potatoes, vegetables, sugar beets, fruit, cotton, hops and turf (EXTOXNET, 2009).

Imidacloprid is currently sold worldwide under several commercial names including, Admire, Chinook Blue and Tripod Plus (Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Imidacloprid is highly soluble in water (610 mg/L) and, in assessing the sorption to soil, a Koc value of 225 L kg-1 has been described suggesting moderate mobility in the environment (Lewis et al., 2007).

Imidacloprid is not readily degraded in soil under aerobic conditions, with degradation half-lives of 187 and 174 days under laboratory (20oC) and field conditions respectively; imidacloprid is considered to be persistent (Lewis et al., 2007).

2.2.2 Metabolite: 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine

1-(6-Chloro-pyridine-3-methyl)-N-nitro guanidine is one of the metabolites formed through degradation of imidacloprid.

No information relating to either the physicochemical properties or environmental fate of the metabolite was found.

2.3 Potential routes of human exposure

Occupational exposure to imidacloprid may occur through inhalation and dermal contact with this compound at workplaces where it is produced or used. The general

population may be exposed to imidacloprid via dermal contact with this compound (HSDB, 2006).

Although no data on routes of exposure of humans to the metabolite 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of imidacloprid, and, to a limited extent, when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics of parent and metabolite

No information is available on the toxicokinetics of imidacloprid in humans.
Toxicokinetic studies have shown that, following oral dosing of [pyridinyl-14C-methylene]imidacloprid at 1 or 20 mg/kg bw to rats, the radiolabel is rapidly absorbed and distributed via plasma around the body. In a further study in rats at a dose of 1 mg/kg bw (i.v.), up to 96% of administered radiolabel was recovered in urine and faeces (ratio 4:1) within 48 hours (WHO/FAO, 2001).

Following a single oral dose of 20 mg/kg bw to male rats, whole body autoradiograph showed that absorbed [pyridinyl-14C-methylene] imidacloprid was readily absorbed and rapidly distributed to tissues and organs within one hour of oral dosing. High levels of radioactivity were seen in kidneys over the first 24 hours, reflecting the high rate of urinary excretion. The main renal metabolite (about 30%) was identified as the glycine conjugate of 6-chloronicotinic acid. Parent compound was also present at about 12%, two monohydroxylated biotransformation products (4-hydroxy and 5-hydroxyimidacloprid) at about 19 % and an olefinic metabolite at about 11%. 6-Chloronicotinic acid represented 7.9% of the renal radiolabel (WHO/FAO, 2001).

No information on the toxicokinetics of 1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine was identified.

4 Toxicity Profile

4.1 Imidacloprid

4.1.1 Acute Toxicity

Two adult deaths have been reported following ingestion of imidacloprid (dose unspecified). However, ingestion of up to 200 mg imidacloprid by a four year old child did not result in any signs of adverse health effects (HSDB, 2006).Experimentally, imidacloprid has moderate acute toxicity in rats; LD50's are 131 mg/kg, 5000 mg/kg and 0.069 mg/m3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007).

In animals, imidacloprid is mildly irritating to skin and eye. It does not appear to be a skin sensitiser (PSD, 1993).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of imidacloprid in humans was identified.
Experimentally, Wistar rats fed imidacloprid in diet at concentration of up to 1800 ppm for 2 years showed decreased body weight gain in females at 300 ppm and increased thyroid lesions in males at 300 ppm and females at 900 ppm. A NOEL of 100 ppm (equivalent to 5.7 and 7.6 mg/kg bw/day in males and females respectively) was established (CEPA, 2004).

In a one-year feeding study, dogs fed a diet at levels up to 2500 ppm showed increased blood cholesterol levels and some hepatic changes (elevated liver cytochrome P450 level). A NOEL of 500 ppm was established (CEPA, 2004).

4.1.3 Carcinogenicity and genotoxicity

No information on the carcinogenic or genotoxic potential of imidacloprid in humans was identified.

In the two year feeding study in rats described above, no carcinogenic effects were reported (CEPA, 2004).

The genotoxicity of imidacloprid has been assessed in a battery of 23 assays; negative results were obtained in all but two. Imidacloprid was positive in a human lymphocyte chromosomal aberration assay and in Chinese hamster ovary cells; imidacloprid may therefore be considered as weakly mutagenic (EXTOXNET, 2009).

4.1.4 Reproductive and development toxicity

No information was found on the reproductive and developmental potential of imidacloprid in humans.

In a three-generation study in rats fed diet containing imidacloprid at up to 700 ppm, body weight gain was reduced in F0 males at 700 ppm. Blood chemistry changes were noted in F1 offspring including increased O-demethylase activity at 250 ppm in females and 700 ppm in both sexes, N-demethylase activity and cytochrome P450 activity at 700 ppm in males. No treatment-related effects on reproductive parameters (fertility index, litter size) were noted and a reproductive toxicity NOEL of 700 ppm was established. However, decreased weight gain was seen in pups at 700 ppm. A developmental toxicity NOEL of 250 ppm was therefore established (CEPA, 2004).

4.2 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine

4.2.1 Acute Toxicity

No information on the acute effects of 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine in humans or animals was found.

4.2.2 Repeat dose toxicity

Searches were made for published information on the toxicity of 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine, including use of the online programme ChemIDPlus (US NLM, 2003). However, no information was found on the effects of repeated exposure to 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine in humans or animals.

Additional information was, therefore, sought through use of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Analysis by DEREK identified the presence of two structural alerts and the following conclusions were drawn:

it is considered plausible (i.e. there is a weight of evidence) that 1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine will be carcinogenic in humans. This endpoint is predicted because 1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine is an N-Nitro or N-nitroso compound.

it is considered Open (i.e. there is uncertain evidence) that 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine will be mutagenic in humans. This endpoint is predicted

because 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine is an N-Nitro or N-nitroso compound.

The analysis by TOPKAT suggested that 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine was:

- non-carcinogenic; but
- mutagenic; and
- a developmental toxicant.

Table 1: Predicted toxicity data for 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$ (range)				
Imidacloprid (experimental data)	131	0.069	NOAEL: 5.7 mg/kg/day (rats) Target organ: thyroid	Weakly mutagenic	Negative	Decreased pup weight gain
1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine (TOPKAT)	213.4 (35.5-1300)	683.8 (101-4600)	MTD (feed/drink) 64.2 mg/kg MTD (oral gavage) 177.7 mg/kg	mutagen	Negative	Developmental toxicant
1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine (DEREK)	n/a	n/a	No alert	*Open* for mutagenicity in humans	*Plausible* in humans	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software; UE –unreliable estimate

5 Guidelines and Standards

5.1 Imidacloprid

The EC risk classification is: Xn - Harmful: R22; N - Dangerous for the environment: R50, R53. The EC Safety classification is S2, S22, S57, S60, S61. Imidacloprid is classified by WHO as 'moderately hazardous' and by the US EPA as 'moderately toxic' (Lewis *et al*., 2007).

An ADI of 0.06 mg/kg bw/d has been established for imidacloprid using a safety factor (SF) of 100, based on a NOAEL of 5.7 mg/kg bw/day from a 2-year rat feeding (Lewis *et al*., 2007).

5.2 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine

No ADI has been proposed by any authoritative organisation for 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

Overall, the toxicity profile for 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine is predicted to be similar to that of the parent. The lowest predicted oral MTD for 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine by TOPKAT is 64.2 mg/kg bw/day (for dietary or drinking water administration). Applying a SF of 100 would give a nominal value of 0.64 mg/kg bw/day which is ten-fold higher than the established ADI of 0.06 mg/kg bw/day for the parent imidacloprid. Given this, it is – on a highly precautionary basis – therefore proposed to adopt a PSDV of 0.06 mg/kg bw/day for 1-(6-chloro-pyridine-3-methyl)-N-nitro guanidine, i.e. the same value as for the parent; this would give an overall safety factor of 2600.

6 References

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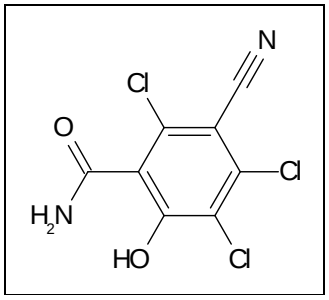
Appendix 12.30 (3-Cyano-6-hydroxy-2,4,5-trichlorobenzamide)

1 Introduction

3-Cyano-6-hydroxy-2,4,5-trichlorobenzamide is a metabolite of the fungicide chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile; CAS No. 1897-45-6).

The structure of 3-Cyano-6-hydroxy-2,4,5-trichlorobenzamide is presented in Figure 1.

Figure 1: Structure of 3-cyano-6-hydroxy-2,4,5-trichlorobenzamide



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Chlorothalonil

Chlorothalonil is a broad-spectrum non-systemic pesticide that is used primarily as a fungicide and mildewicide but has some additional activity as a bactericide, microbiocide, algaecide, insecticide and acaricide. Chlorothalonil was first registered in the US in 1966 for application to turf but is now registered for a wide range of applications including on fields, vegetables and orchard crops and as a mildewicide in paint and other surface treatments (US EPA, 1999).

Chlorothalonil is currently sold worldwide under several commercial names including Bravo, Daconil 2787, Echo, Exotherm Termil, Forturf, Mold-Ex, Nopcocide N-96, Ole, Pillarich, Repulse and Tuffcide. In general it is sold as a soluble concentrate that is mixed with water and applied as a spray. Application rates vary depending on use. For most crops the maximum rate of application is between 0.28 – 0.41 kg chlorothalonil per hectare although two crops require a higher rate of 0.83-0.92 kg chlorothalonil per hectare (ENVIROfacts, 2003).

2.2 Environmental fate

2.2.1 Parent

Any chlorothalonil released into the air during application is likely to enter both vapour and particulate phases. Chlorothalonil within the vapour phase will be slowly photochemically degraded with an estimated half-life of 7 years (HSDB, 2006).

Although photolysis might also occur in the particulate phase, no data on the rate at which this might occur is available. Particulate-phase chlorothalonil is mainly removed from the atmosphere by wet and dry deposition.

Chlorothalonil has low water solubility (0.81 mg/L at 25oC, pH (neutral); EC, 2006). Koc values of between 900 and 7000 L kg-1 have been described (HSDB, 2006) suggesting slight or no mobility to soils.
Chlorothalonil is moderately susceptible to degradation in soil under aerobic conditions (US EPA, 1999). Degradation occurs mainly through dechlorination and some substitution reactions (Sato et al., 1987). The degradation half-life in four soils

has been reported to be between 10 and 40 days (HSDB, 2006); the average half-life was 22 days (Lewis et al., 2007). Chlorothalonil is not considered to be persistent.

2.2.2 Metabolite: 3-Cyano-6-hydroxy-2,4,5-trichlorobenzamide
No information on the physical properties or environmental fate of 3-Cyano-6-hydroxy-2,4,5-trichlorobenzamide was identified.

2.3 Potential routes of human exposure
During production Chlorothalonil may be released to the environment through various waste streams. Direct release into the environment will also occur during its use as a pesticide. Exposure to chlorothalonil may occur through ingestion of contaminated food and water or through inhalation or dermal contact during occupational handling or by-stander exposure (HSDB, 2006).

Although no data on routes of exposure of humans to the metabolite SDS-4685 was identified, it is plausible that exposure may occur as a result of its ingestion in food and water contaminated from the breakdown of chlorothalonil (oral route) or when formed as a human metabolite from ingestion of the parent compound.

3 Toxicokinetics
No information is available on the toxicokinetics of chlorothalonil in humans.

Toxicokinetic studies have been carried in Sprague-Dawley rats orally dosed with 14[C]-chlorothalonil at 5, 50 or 200 mg/kg. In males, approximately 89% of the radioactive dose was excreted and in females approximately 96%. The major route of excretion was the faeces (83 - 87%) with excretion apparently complete within 48 hr in low dose females and low and mid dose males, and by 72 hr in mid/high dose females and high dose males. Some urinary excretion also occurred which showed saturation at higher doses. At low doses, urinary excretion was 92 - 93% complete within 24 hr, and for mid doses within 48 hr, however, at high doses only 95% excretion was achieved by 72 hr (IPCS, 1996).

A similar pattern was noted in rats given a single low oral dose (1.5mg/kg) of chlorothalonil. Around 30 - 32% of administered dose was absorbed from the GI tract, with approximately 20 - 22% of the absorbed dose being excreted in bile, and a further 10% excreted in urine (Krieger, 2001).

In monkeys treated dermally with 5 mg/kg bw of 14[C]-chlorothalonil under a non-occlusive patch for 48 hr, around 90% of the dose was recovered from the treated surface, i.e. unabsorbed; only 2.26% was absorbed through the skin. Urine contained 1% of the absorbed dose but this did not include any detectable methylated mono-, di- and trithiols (IPCS, 1996). In male Sprague-Dawley rats given 200 mg/kg 14[C]-chlorothalonil by oral gavage, urine collected at 17, 24 and 48 hr following administration contained the metabolites trimethylthiomonochloro-isophthalonitrile and dimethylthiodichloro-isophthalonitrile, excreted as free thiols or methylated derivatives, and accounted for 2.4% of the administered dose (IPCS, 1996).

In a repeat oral dose study male rats were given 14[C]-chlorothalonil at 1.5, 5, 50 or 160 mg/kg/day, and culled 2, 9, 24, 96 and 168 hr following administration of the last dose. Chlorothalonil was found to be widely distributed, with highest concentrations in the kidneys followed by liver and blood. Peak concentrations were seen 2 hr after the last dose, at which time 0.28% and 0.20% of the radioactivity was found in the kidney for the 1.5 and 5 mg/kg dose levels respectively (IPCS, 1996).

In a further study, rats were given repeated doses of 14[C]-chlorothalonil at up to 160 mg/kg/day. Methylated or partly methylated dithiol and trithiol derivatives were identified in urine at all doses (IPCS, 1996).

No information on the toxicokinetics of 3-Cyano-6-hydroxy-2,4,5-trichlorobenzamide was identified.

4 Toxicity Profile
4.1 Chlorothalonil
4.1.1 Acute Toxicity
No information is available on the acute toxicity of chlorothalonil to humans.

Chlorothalonil has low acute toxicity in rats; LD50's are 5000 mg/kg, 2000 mg/kg and 0.1 mg/m3 when given by oral, dermal or inhalation routes respectively (EC, 2006).
Chlorothalonil is a contact irritant to the skin, eye and respiratory tract (US EPA, 1999) and there is evidence to suggest that it is a skin sensitiser (EC, 2006).

4.1.2 Repeat dose toxicity
Repeated exposure of humans to chlorothalonil has been associated with development of contact dermatitis and other skin conditions. For example, employees in a chlorothalonil manufacturing plant were reported to have an increased incidence (60%) of skin abnormalities compared with non-exposed workers (18.5%; IPCS, 1996).

In animal studies, the principal target organs of repeated exposure are considered to be the kidney and forestomach.

In a 2 year feeding study, Fischer-344 rats were administered chlorothalonil via the diet at up to 175mg/kg bw/day. Histopathological examination of the kidneys showed nephropathy, tubular hyperplasia (considered a preneoplastic change) and chronic progressive nephropathy in rats of treated groups. Increased incidence and severity of hyperplasia and hyperkeratosis of the squamous mucosa of the oesophagus and forestomach were also noted in treated groups; the stomach and oesophageal changes were attributed to an irritant effect of chlorothalonil. A NOEL of 1.8 mg/kg bw/day was established for non-neoplastic effects (IPCS, 1996).

Similar findings were noted in Beagle dogs administered chlorothalonil at dietary levels up to 750 mg/kg bw/day for 2 years, with treatment-related histopathological changes being seen in the liver, thyroid, kidney and stomach at mid and high doses (IPCS, 1996).

4.1.3 Carcinogenicity and mutagenicity
There is inadequate evidence of carcinogenicity of chlorothalonil in humans. However, IARC has noted that there is sufficient evidence of carcinogenicity in experimental animals, and concluded that chlorothalonil is possibly carcinogenic to humans (Group 2B; IARC, 1999).

In the two year dietary study in which Fischer-344 rats were administered chlorothalonil at up to 175 mg/kg bw/day described above, the incidence of renal tubular adenoma and carcinoma was increased in treated male and female groups. A treatment related increase in incidence and severity of renal tubular epithelial hyperplasia was also noted and was considered a pre-neoplastic change. Hyperplasia and hyperkeratosis of the squamous mucosa of the oesophagus and forestomach were also apparent (IPCS, 1996).

The carcinogenic potential of chlorothalonil administered in the diet at doses up to 165 mg/kg bw/day was assessed in a further rat study; a NOEL of 3.8 mg/kg bw/day was established (IPCS, 1996).

Evidence on the genotoxic potential of chlorothalonil is conflicting. Although some in vitro models without activation have shown positive results, the available in vivo studies were inconclusive (EC, 2006; HSDB, 2006).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of chlorothalonil in humans was identified.

In a two-generation reproduction study in Charles River CD rats, chlorothalonil was administered via the diet at up to 3000 mg/kg diet. No treatment-related effects were noted in F0 or F1 parent animals and reproductive parameters were unaffected. However pups displayed lower bodyweights. A NOEL for this effect of 75 mg/kg bw/day was proposed (IPCS, 1996).

Similar results have been described in rabbits when given chlorothalonil by oral gavage at up to 20 mg/kg/day on days 7 - 19 of gestation. A lowered maternal body weight in conjunction with decreased food consumption was noted at the highest dose; pregnancy rate was > 95% in all groups and no evidence of fetotoxicity or teratogenicity was found (IPCS, 1996).

4.2 3-Cyano-6-hydroxy-2,4,5-trichlorobenzamide

Searches were made for published information on the toxicity of 3-Cyano-6-hydroxy-2,4,5-trichlorobenzamide, including use of the online programme ChemIDPlus (US NLM, 2003). However, no information was found.

Additional information was, therefore, sought through use of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1: Predicted toxicity data for 3-cyano-6-hydroxy-2,4,5-trichlorobenzamide using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/ H (range)				
Chlorothalonil (experimental data)	5000	0.1	NOAEL: 1.5 mg/kg/day Main target organs: kidney and fore-stomach	Genotoxic potential not established	Fore-stomach tumours in rats and mice, kidney tumours in mice	Reduced pup weight (rats).
3-Cyano-6-hydroxy-2,4,5-trichlorobenz-amide (TOPKAT)	33.5 (6.9-162.5)	352.4 (41.3-3000)	MTD (feed/drink) 1000 mg/kg MTD (oral gavage) 1000 mg/kg	Negative	Negative	Non-developmental toxicant
3-Cyano-6-hydroxy-2,4,5-trichlorobenz-amide (DEREK)	n/a	n/a	*Plausible* for photoallergenicity in humans	No alert	*Equivocal* for carcinogenicity in humans	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software

Analysis by DEREK identified a number of structural alerts in the molecule and the following conclusions were drawn:

it is considered plausible (ie there is a weight of evidence) that 3-Cyano-6-hydroxy-2,4,5-trichlorobenzamide will exhibit photoallergenicity in some animals (Dog, Guinea Pig, Hamster, Human, Mammal, Monkey, Mouse, Primate, Rabbit, Rat, Rodent). This endpoint is predicted because 3-Cyano-6-hydroxy-2,4,5-trichlorobenzamide is a halogenated aromatic compound.

it is considered equivocal that 3-Cyano-6-hydroxy-2,4,5-trichlorobenzamide will exhibit carcinogenicity in some animals (Dog, Guinea Pig, Hamster, Human, Mammal, Monkey, Mouse, Primate, Rabbit, Rat, Rodent). This endpoint is predicted because 3-Cyano-6-hydroxy-2,4,5-trichlorobenzamide is a polyhalogenated aromatic compound.

The analysis by TOPKAT suggested that 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid was:

non-carcinogenic;
non-mutagenic; and
a non-developmental toxicant.

5 Guidelines and Standards

5.1 Chlorothalonil

The EC risk classification for chlorothalonil is: Carcinogen category 3: R40; T+ - very toxic: R26; Xi – Irritant: R37, R41, R43; N – Dangerous for the environment: R50, R53. Chlorothalonil is classified by WHO as 'unlikely to present acute hazard in normal use', and by the US EPA as 'moderately toxic' (Lewis et al., 2007).

An ADI of 0.018 mg/kg bw/d has been established for chlorothalonil using a safety factor (SF) of 100, based on a two year rat study in which Fischer 344 rats received chlorothalonil via the diet at up to 175 mg/kg/day (IPCS, 1996; Lewis *et al*., 2007).

5.2 3-Cyano-6-hydroxy-2,4,5-trichlorobenzamide

No experimental data is available on 3-Cyano-6-hydroxy-2,4,5-trichlorobenzamide and no ADI has been proposed by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The repeat dose toxicity profile for 3-Cyano-6-hydroxy-2,4,5-trichlorobenzamide is overall predicted to be somewhat less than that of the parent, with an oral MTD predicted by TOPKAT 1000 mg/kg bw (irrespective of type of oral administration). Applying a nominal SF of 100 would give a nominal value of 10 mg/kg bw which is considerably higher than the established ADI of 0.005 mg/kg/bw for the parent, chlorothalonil. Given this, it is – on a highly precautionary basis –proposed to adopt a PSDV of 0.005 mg/kg bw/d for 3-Cyano-6-hydroxy-2,4,5-trichlorobenzamide, i.e. the same value as for the parent; this will provide an overall SF of >10,000.

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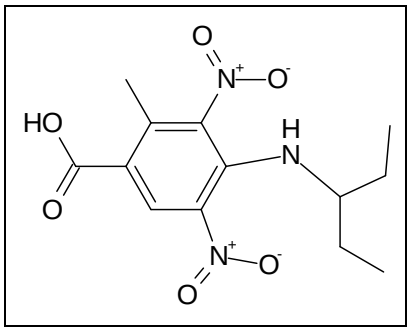
Appendix 12.31 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid

1 Introduction

4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid is a metabolite of the herbicide pendimethalin (*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine; CAS No. 40487-42-1).

The structure of 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol is presented in Figure 1.

Figure 1: Structure of 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Pendimethalin

Pendimethalin is a selective herbicide used pre- and post-emergence to control annual grasses and annual broad-leaved weeds in field corn, potatoes, rice, cotton, soybeans, tobacco, peanuts and sunflowers (HSDB, 2003). Pendimethalin is sold worldwide under the trade names, Blazer, Bunker, Claymore, PDM 330 EC, PicoMax, Picona, PicoPro, Stomp, PicoStomp, Pendimethalin 330 EC and Trump (Lewis et al., 2007).It is often supplied as an emulsifiable or emulsion concentrate that is mixed with water and used as a spray (Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Pendimethalin has low solubility in water (0.33 mg/L at 20oC). In assessing the sorption to soil, a Koc value of 15744 l/ kg has been reported suggesting that it is not mobile in the environment (Lewis et al., 2007).

Studies have shown pendimethalin is moderately degraded in soils with half-lives of 123 and 90 days, under laboratory (20oC) and field conditions respectively. The compound is moderately persistent in the environment (Lewis et al., 2007).

During use, pendimethalin will be released into the atmosphere where it will be present in the vapour phase where it can react with photochemically-produced hydroxyl radicals; the estimated atmospheric half-life of 12.7 hr (HSDB, 2003).

2.2.2 Metabolite: 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid

4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid is one of the metabolites formed following degradation, or to a limited extent, metabolism of pendimethalin.

No information on the physicochemical properties or environmental fate of 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid was identified.

2.3 Potential routes of human exposure
Exposure of humans to pendimethalin will primarily be through occupational dermal contact and inhalation and ingestion of aerosols during mixing and application and by contact with treated plants and soil. The general population is most likely to be exposed to pendimethalin through ingestion of and dermal contact with contaminated water, including rain water. This is most likely to occur near agricultural areas during the growing season (HSDB, 2003).

Although no data on routes of exposure of humans to the metabolite 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of pendimethalin, and, to a limited extent, when formed as a human metabolite following ingestion of the parent compound

3 Toxicokinetics
No information is available on the toxicokinetics of pendimethalin in humans.

Experimentally, the toxicokinetics of pendimethalin was established in a single oral dose study in which rats were given 7.3 and 37 mg/kg 14[C]-pendimethalin. Absorption from the GI tract was shown to be very low and a large proportion of pendimethalin was excreted unchanged in the faeces. Rapid metabolism of absorbed pendimethalin occurred in the kidneys and liver, with metabolites excreted in urine. Following a period of 24 hr after administration of a 37 mg/kg dose, over 90% was seen to be recovered in faeces and urine, with 96% recovery after 4 days (Zulalian, 1990).

Tissue distribution of absorbed pendimethalin was found to be minimal with 0.3 ppm being recovered in all tissues and 0.9 ppm in fat 4 days following administration (Zulalian, 1990).

Metabolism of pendimethalin was shown to involve hydroxylation of the 4-methyl and the N-1-ethyl group, oxidation of these alkyl groups to carboxylic acids, nitro reduction, cyclization, and conjugation (Zulalian, 1990).

No information on the toxicokinetics of 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid was identified.

4 Toxicity Profile
4.1 Pendimethalin
4.1.1 Acute Toxicity
No information is available on the acute toxicity of pendimethalin in humans.

In rats, pendimethalin has moderate acute toxicity; LD50's are 3189 mg/kg, 2000 mg/kg and 320 mg/m3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007).

Pendimethalin is not a skin irritant or sensitizer in rabbits or guinea pigs, but causes mild eye irritation in rabbits. Inhalation of dusts or fumes may be mildly to moderately irritating to the linings of the mouth, nose, throat, and lungs (EXTOXNET, 1996).

4.1.2 Repeat dose toxicity
No information on the repeat dose toxicity of pendimethalin in humans was identified.
Experimentally, the principal target organs of repeated exposure for pendimethalin are the liver and, in rats only, the thyroid (EC, 2003).

In Beagle dogs fed for two years on diets containing pendimethalin at up to 200 mg/kg bw/day, serum alkaline phosphatise (SAP) activity was increased at the two highest doses. Increased liver weight and associated inflammatory changes and haemosiderosis were also noted in the two highest dose groups. A NOEL of 12.5 mg/kg/day was established, based on the hepatic toxicity (IRIS, 1991).

In rats fed pendimethalin in the diet for 56 days for study of thyroid function only, exposure to 500 ppm (31 mg/kg bw/day) caused changes in serum chemistry by day 28 ; these comprised statistically significant decreases (38%) in total serum T4, reverse T3 (25%) and total T4 (28%) and an increase in free T3 (13%).

4.1.3 Carcinogenicity and mutagenicity

No information on the carcinogenic and mutagenic potential of pendimethalin in humans was identified.

In the 56 day rat thyroid function study reported above, histopathological changes included increased thyroid follicular cell height (40%) and decreased colloid areas (37%); some of these changes were detectable by day 3 of treatment. Although such changes may be indicative of carcinogenic potential, no tumour diagnosis was apparent in the study. However, a NOEL of 100 ppm (10 mg/kg/day) was established for potential carcinogenic effects (CEPA, 1999) and pendimethalin has been classified by the US EPA as a Group C, possible human carcinogen (US EPA, 1997).

In mice fed a diet containing 75 mg/kg bw/day of pendimethalin for 18 months, no evidence of increased tumour formation was reported (EXTOXNET, 1996).

Experimentally, pendimethalin was negative in a number of in vitro and in vivo mutagenicity assays including tests on live animals and mammalian and bacterial cell cultures (EXTOXNET, 1996).

4.1.4 Reproductive and development toxicity

No information was identified on the reproductive or developmental effects of pendimethalin on humans.

Experimentally, in a three-generation rat study in which pendimethalin was administered via the diet at up to 250 mg/kg/day, reduced offspring numbers at birth and offspring growth (from weaning to maturity) were noted at the mid and high doses. No effects were observed below 30 mg/kg/day, and a reproductive NOEL of 25 mg/kg/day was established (IRIS, 1991).

Teratology studies in pregnant rats administered pendimethalin in the diet at up to 500 mg/kg bw/day have been reported. No fetotoxic or teratogenic effects were noted and a NOAEL of 500 mg/kg bw/day was established (IRIS, 1991).

4.2 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid

No information on the repeat dose toxicity of 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid in humans was identified.

Searches were made for publicly available information on the toxicity of 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid, and by use of the online programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1 Predicted toxicity data for 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Pendimethalin (experimental data)	3189	320	NOAEL: 12.5 mg/kg/day Main target organs: Liver and thyroid (rats)	Negative	Some evidence in rats (thyroid).	Negative
4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid (TOPKAT)	3300 (748.9-10,000)	10,000 (2000-10,000)	MTD (feed/drink) 265.4 mg/kg MTD (oral gavage) 265.4 mg/kg	Negative	Negative	Negative
4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid (DEREK)	n/a	n/a	*Plausible* for Hepatotoxicity in humans	*OPEN* (mutagenicity) and *Equivocal* (genotoxicity) in humans	*Plausible* in humans	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software; UE- unreliable estimate

Analysis by DEREK identified the presence of a number of structural alerts in the molecule, and the following conclusions were drawn:

It is considered plausible (i.e. there is a weight of evidence) that 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid will exhibit carcinogenicity in humans. This endpoint is predicted because 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid is an aromatic nitro compound;

It is considered plausible (i.e. there is a weight of evidence) that 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid will exhibit hepatotoxicty in humans. This endpoint is predicted because 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid is an aromatic nitro compound; and

It is considered OPEN (i.e. there is no overall weight of evidence) that 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid will exhibit mutagenicity in humans. This endpoint is predicted because 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid is an aromatic nitro compound.

It is considered equivocal (i.e. there is uncertain evidence) that 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid will exhibit chromosome damage in some humans. This endpoint is predicted because 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid is an aromatic nitro compound

The analysis by TOPKAT suggested that 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid was:

non-carcinogenic;
non-mutagenic; and
not a developmental toxicant.

5 Guidelines and Standards

5.1 Pendimethalin

The EC risk classification is: Xi - Irritant: R43; N - Dangerous for the environment: R50, R53 and EU safety category is S2, S22, S29, S37, S60, S61. Pendimethalin is classified by WHO as 'slightly hazardous' and the US EPA as 'slightly toxic' (Lewis *et al.,* 2007).

An ADI of 0.125 mg/kg bw/day was established for pendimethalin (EU, 2003) using a safety factor (SF) of 100, based on the two feeding study in Beagle dogs administered pendimethalin at up to 200 mg/kg bw/day (Lewis *et al*., 2007).

5.2 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid

Due to the lack of availability of experimental data, it has not been possible to establish a robust NOEL or NOAEL for 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid, and no ADI has been proposed by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The general repeat dose toxicity profile for 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid is overall predicted to be somewhat less than that of the parent. The highest oral MTD predicted by TOPKAT for 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid is 265.4 mg/kg bw (irrespective of method of oral administration). Applying a SF of 100 would give a nominal value of 2.65 mg/kg bw/day, which would be considerably higher than the established ADI of 0.125 mg/kg bw/day for the parent pendimethalin. However, given the predicted hepatotoxicity and the concerns regarding potential mutagenic and carcinogenic potential by one of the two predictive systems utilised, it is therefore proposed to adopt a PSDV of 0.125 mg/kg bw/day for 4-[(1-Ethylpropyl)amino]-3,5-dinitro-o-toluic acid (i.e. the same value as for the parent); this will provide an overall SF of greater than 2000 .

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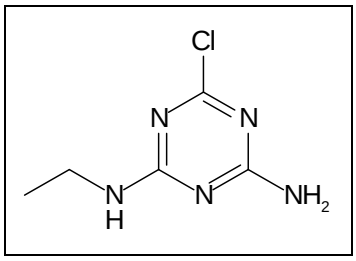
Appendix 12.32 Desisopropylatrazine

1 Introduction

Desisopropylatrazine (CAS No. 1007-28-9) is a metabolite of the herbicide simazine (6-chloro-*N*,*N*'-diethyl-1,3,5-triazine-2,4-diamine; CAS No. 122-34-9). It is formed within the surrounding soil after application of the parent to plants, and during metabolism of absorbed simazine by humans.

The structure of desisopropylatrazine is presented in Figure 1.

Figure 1: Sturucture of desisopropylatrazine



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Simazine

Simazine is a pre-plant herbicide that is used worldwide on a variety of food and feed crops including fruit and nut crops (e.g. apples, oranges and almonds) and corn. Simazine can also be applied at forestry sites and on turf grass grown commercially for sod. The herbicide also has non-agricultural uses as a non-selective weed control on non-crop land such as industrial sites, roadsides and railways. Simazine is also registered for residential use on turf grass including commercial use on recreational lawns such as golf courses, and commercial or homeowner use on home lawns. In addition, simazine is used as an algaecide in ornamental ponds and aquariums (HSDB, 2007).

2.2 Environmental fate

2.2.1 Parent

Simazine has low solubility in water (5 mg/L) and, in assessing the sorption to soil, a Koc value of 130L kg-1 has been described suggesting moderate mobility in the environment (Lewis et al., 2007).

Simazine is moderately susceptible to degradation in soil under aerobic conditions with a degradation half-life of 90 days under field conditions. Simazine is considered to be moderately persistent (Lewis et al., 2007).

2.2.2 Metabolite: Desisopropylatrazine

Desisopropylatrazine is one of the metabolites formed through degradation and metabolisms of Simazine (Lewis et al., 2007).

Desisopropylatrazine has high solubility in water (670 mg/L) and, in assessing the sorption to soil, a Koc value of 142L kg-1 has been described suggesting moderate mobility in the environment (Lewis et al., 2007).

No further information on the physicochemcial properties or environmental fate of desisopropylatrazine was found.

2.3 Potential routes of human exposure

Occupational exposure to simazine may occur through inhalation of dust particles and dermal contact during or after its application or at workplaces where it is produced or used. The general population may be exposed via dermal contact, inhalation of ambient air or through ingestion of contaminated drinking water and food (HSDB, 2007).

Exposure of humans to the metabolite Desisopropylatrazine may occur through ingestion of this metabolite in food and water contaminated with Simazine and its metabolites or through during the metabolism of any absorbed parent herbicide.

Although no data on routes of exposure of humans to the metabolite desisopropylatrazine was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of simazine, and, to a limited extent, when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics of parent and metabolite

No information on the toxicokinetics of simazine in humans was identified.

In rats given a single oral dose of radiolabeled simazine at 0.5 or 100 mg/kg bw, around 90% was absorbed at the low dose whereas only 65% was absorbed at the high dose. Radiolabel was distributed throughout the body with the highest levels occurring in erythrocytes; heart, lung, spleen, kidney and liver also showed a high degree of retention. Overall, 94 - 99 % of radioactivity was eliminated within 48 to 72 hours. The half-life of elimination was 9 - 15 hours. Metabolism involved removal of alkyl side chains and conjugation of the triazine ring with glutathione-S-transferase. The major metabolites in rats were mono- and di-dealkylated compounds, 2-chloro-4-ethylamino- 6-amino-s-triazine and diaminochlorotriazine (DACT). Conjugated mercapturates of hydroxylsimazine were also detected (US EPA, 2005). Routes of excretion were dose-dependent. At a low dose, the principal route of excretion was urine (63%) with lesser amounts excreted in faeces (25%). However, at the highest dose, the major route of excretion was faeces (49%) with urine accounting for 39% (Bingham, 2001).

No information on the toxicokinetics of desisopropylatrazine was identified.

4 Toxicity Profile

4.1 Simazine

4.1.1 Acute Toxicity

Acute exposure of humans to simazine may cause rashes and dermatitis. Other symptoms following acute exposure at high doses include difficulty in walking, tremor, convulsions, paralysis, cyanosis, slowed respiration, miosis (pinpoint pupils), gut pain, diarrhoea, and impaired adrenal function (HSDB, 2007).

Experimentally, simazine has low acute toxicity in rats; LD50's are 5000 mg/kg, 2000 mg/kg and 5.5 mg/m3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007).

Simazine is non-irritating to the skin and eyes of rabbits except at high doses (Briggs, 1992) and patch tests on humans have shown that simazine is not a skin irritant, fatiguing agent or sensitiser (Briggs, 1992).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of simazine in humans was identified.

In a one-year feeding study, Sprague- Dawley rats were administered simazine in diet at up to 1000 ppm (equivalent to 45.8 and 63.1 mg/kg bw/day, for males and females respectively). A significant decrease in body weight and body weight gain was noted at the highest dose, which was associated with lower food consumption. Haematological and blood chemistry effects were limited but included, at the highest dose, a significant increase in mean corpuscular haemoglobin concentration in males and decreased serum glucose level in females. At 1000 ppm, heart, kidney and liver weights were increased in females as were liver and testes weights in males. Histopathological examination showed in females, significantly increased liver haematopoiesis, splenic haematopoiesis and cystic mammary glandular hyperplasia for the high dose group. A LOEL of 100 ppm (equivalent to 4.2 and 5.3 mg/kg bw/day for males and females respectively) and a NOEL of 10 ppm (0.41 and 0.52 mg/kg bw/day for males and females respectively) were established based (US EPA, 2005).

4.1.3 Carcinogenicity and genotoxicity

No information on the carcinogenic or genotoxic potential of simazine in humans was identified.

In the one-year feeding study in rats described above, an additional group of rats were administration simazine for up to 2 years to assess carcinogenicity. In females, increases in incidences of mammary, pituitary and kidney neoplasms were seen. Mammary tumours comprised mammary carcinoma and fibroadenoma, both of which were significantly increased at the high dose, while mammary carcinoma incidence was also significantly increased for mid-dose female. In males a non-significant increase in liver tumours (combined adenoma and carcinoma) was reported (US EPA, 2005).

The mutagenicity of simazine has been assessed in a number of mutagenicity assays but results are inconclusive. Simazine was negative in bacterial assays (US EPA, 1988) while assays using human lung cell cultures have produced both positive and negative findings (US EPA, 1988). In a sex-linked mutation assay in Drosophila a positive finding was noted when adult male flies were dosed but this was not noted when the treatment was given at the larval stage (Stevens, 1991).

IARC considered that there was inadequate evidence in humans for the carcinogenicity of simazine, and only limited evidence in experimental animals; simazine was classified as ‘not classifiable as to its carcinogenicity to humans’ (Group 3; IARC, 1999).

4.1.4 Reproductive and development toxicity

No information was found on the reproductive and developmental effects of simazine in humans.

In a two-generation reproductive toxicity study, Sprague-Dawley rats were given diet treated with simazine at up to 500 ppm. Decreased body weight and body weight gain was noted in F0 males at 1000 or 100 ppm, in F1 males at 1000 ppm and F0 and F1 females at 1000 ppm. Decreased food consumption was also noted at the highest dose in both sexes of the F0 and F1 generations. No reproductive changes were observed. A NOAEL for reproductive toxicity of 500 ppm (equivalent to 31.93 mg/kg bw/day) was established for both sexes (US EPA, 2005).

In pregnant CR1 rats dosed orally at up to 600 mg/kg/day from day 6 to 15 of gestation, maternal toxicity was seen. This comprised decreased body weight, body

weight gains and food utilisation efficiency in the mid- and high-dose. A significant increase in treatment-related skeletal changes was noted in offspring of these groups reflecting a widespread lack of ossification. The maternal and developmental NOAELs were established as 30 mg/kg/day (US EPA, 2005).

4.2 Desisopropylatrazine

4.2.1 Acute Toxicity

No information on the acute toxicity of desisopropylatrazine in humans or animals was identified.

4.2.2 Repeat dose toxicity

No information on the repeat dose toxicity of desisopropylatrazine in humans was identified.

Searches were made for published information on the toxicity of desisopropylatrazine, and by use of the online programme ChemIDPlus (US NLM, 2003). However, no relevant data was identified.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1 Predicted toxicity data for Desisopropylatrazine using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$ (range)				
Simazine (experimental data)	5000	5.5	NOAEL: 0.47 mg/kg/day (rats)	Possible weak mutagen	Mammary tumours (rats)	Offspring skeletal effects
Desisoprop-ylatrazine (TOPKAT)	2100 (369.7-10,000)	10,000 (2500-10,000)	MTD (feed/drink) 633.1 mg/kg MTD (oral gavage) 1800 mg/kg	Negative	Positive	Negative
Desisoprop-ylatrazine (DEREK)	n/a	n/a	*Plausible* for hepatotox-icity, respiratory and skin sensitisation in humans	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software

DEREK identified a number of structural alerts and the following conclusions were drawn:

it is considered plausible (i.e. there is a weight of evidence) that desisopropylatrazine will be cause hepatotoxicity in humans. This endpoint is predicted because desisopropylatrazine is a 2,4-Diamino-1,3,5-triazine.

it is considered plausible (i.e. there is a weight of evidence) that desisopropylatrazine will cause respiratory sensitisation in humans. This endpoint is predicted because desisopropylatrazine is a halo-diazine or triazine.

it is considered plausible (i.e. there is a weight of evidence) that desisopropylatrazine will cause skin sensitisation in humans. This endpoint is predicted because desisopropylatrazine is an activated N-heterocycle.

The analysis by TOPKAT suggested that desisopropylatrazine was:

carcinogenic; but
non-mutagenic; and
not a developmental toxicant

5 Guidelines and Standards

5.1 Simazine

The EC risk classification is: Carcinogen category 3: R40; N - Dangerous for the environment: R50, R53. The EC Safety classification is S2, S36/37, S46, S60, S61. Simazine is classified by WHO as ‘unlikely to present acute hazard in normal use’ and by the US EPA as ‘not acutely toxic’ (Lewis *et al*., 2007).

An ADI of 0.005 mg/kg bw/day has been established for simazine, based on a one-year rat feeding study for which a NOEL of 10 ppm (0.41 and 0.52 mg/kg bw/day for males and females respectively) was established; a safety factor (SF) of 100 was applied (Lewis *et al*., 2007).

5.2 Desisopropylatrazine

Due to the lack of availability of experimental data, it has not been possible to establish a robust NOAEL for desisopropylatrazine and no ADI has been proposed by any authoritative organisation. Therefore, for the purposes of this study a ‘project specific derived value’ (PSDV) has been derived for use in the risk assessment.

The predictive systems used suggest that desisopropylatrazine may have some of the toxic properties of the parent, but that its overall toxicity may be significantly lower. The lowest oral MTD predicted by TOPKAT for desisopropylatrazine was 633.1 mg/kg bw/day. Applying a SF of 1000 would give a nominal value of 0.63 mg/kg bw/day which is markedly higher than the established ADI of 0.005 mg/kg bw/day for the parent simazine. Given this, it is – on a highly precautionary basis – therefore proposed to adopt a PSDV of 0.005 mg/kg bw/d for desisopropylatrazine, i.e. the same value as for the parent. This would give an additional safety factor of >10,000.

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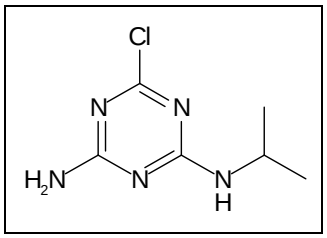
Appendix 12.33 Desethylatrazine

1 Introduction

Desethylatrazine (4-Amino-2-chloro-6-ethylamino-s-triazine; CAS No. 6190-65-4) is a major metabolite of the herbicide atrazine (6-chloro-*N*-ethyl-*N*'-(1-methylethyl)-1,3,5-triazine-2,4-diamine; CAS No. 1912-24-9) that is formed within the surrounding soil; the metabolite is also formed following ingestion of atrazine by humans.

The structure of desethylatrazine is presented in Figure 1.

Figure 1: Structure of desethylatrazine



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of atrazine

Atrazine is a selective triazine herbicide used to control broadleaf and grassy weeds in corn, sorghum, sugarcane, pineapple, Christmas trees, and other crops, and in conifer reforestation plantings. It is also used as a nonselective herbicide on non-cropped industrial lands and on fallow lands (EXTOXNET, 1996).

Atrazine is sold worldwide under the trade names Gesaprim, Fenamin and Atrazinax. In general it is available as dry flowable, flowable liquid, liquid, water dispersible granular, and wettable powder formulations (Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Atrazine has low solubility in water (35 mg/L) and, in assessing the sorption to soil, a Koc value of 100L kg-1 has been described suggesting moderate mobility in the environment (Lewis et al., 2007).

Atrazine is moderately susceptible to degradation in soil under aerobic conditions, with a degradation half-life of 66 and 29 days under laboratory (20oC) and field conditions respectively; atrazine is considered to be moderately persistent (Lewis et al., 2007).

2.2.2 Metabolite: Desethylatrazine

Desethylatrazine is one of the major compounds formed through metabolism or degradation of atrazine (Lewis et al., 2007).

Desethylatrazine has high solubility in water (320 mg/L) and, in assessing the sorption to soil, a Koc value of 72 L kg-1 has been described suggesting that the metabolite will be mobile in the environment (Lewis et al., 2007).

2.3 Potential routes of human exposure

Occupational exposure to atrazine may occur through inhalation and dermal contact at workplaces where it is produced or used. The general population may be exposed

to atrazine via inhalation of ambient air and ingestion of food and drinking water containing the herbicide (HSDB, 2005).

Although no data on routes of exposure of humans to the metabolite desethylatrazine was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of atrazine, and, to a limited extent, when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics of parent and metabolite

The toxicokinetics of atrazine following dermal exposure in humans has been assessed in 10 volunteers given a single topical dose of [triazine ring-U-14C]-atrazine at concentrations of 6.7 or 79 µg/sq cm (equivalent to 0.1667 and 1.9751 mg of [14C]-atrazine for the low and high dose respectively) for 24 hours. The majority (91.1 - 95.5%) of the dose remained unabsorbed and was detected in skin wash samples taken 24 hours after dosing. At both application levels, peak urinary elimination occurred between 24 and 48 hours, with peak faecal elimination during 48 - 72 hours (US EPA, 2003).

Experimentally, the toxicokinetics of atrazine has been studied in rats given a single oral dose of 14[C]-atrazine at up to 100 mg/kg bw. Absorption through the GI tract was complete and consistent urine: faecal excretion ratios were noted across the dose range. At ten days after administration, the highest levels of radioactivity were associated with red blood cells (1.6% of dose) and liver (0.6% of dose). Metabolism of atrazine involved cytochrome P-450 mediated N-dealkylation. 2-Chloro-4,6-diamino-s-triazine was identified as the major metabolite in the rat. Oxidation of the primary position of the alkyl side chains to carboxyl functions also occurred as a minor metabolic route. The major route of excretion was urine (75%) with a lesser amounts excreted via faeces (15%; PSD, 1993).

No information is available on the toxicokinetics of desethylatrazine.

4 Toxicity Profile

4.1 Atrazine

4.1.1 Acute Toxicity

Acute over exposure of humans to atrazine has been reported to cause a range of symptoms including: fatigue; dizziness; nausea; irritation of skin, eyes and respiratory tract; and allergic eczema or asthma. Ingestion of 100 g may lead to coma, circulatory collapse, metabolic acidosis and gastric bleeding; this may be followed by renal failure, hepatic necrosis and a disseminated intravascular coagulopathy which may be fatal (Ellenhorn, 1997).

Atrazine is irritant to the skin and eye (Ellenhorn, 1997).

Experimentally, atrazine has moderate acute toxicity in rats; LD50's are 1869 mg/kg, 3100 mg/kg and 5.8 mg/m3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007).

4.1.2 Repeat dose toxicity

No information on the effects of repeated exposure of humans to atrazine was identified.

Experimentally, male and female rats given atrazine in the diet at up to 100 ppm for two years showed no gross or microscopic signs of toxicity (HSDB, 2005).

Chronic toxicity studies in CD-1 mice given atrazine in diet at up to 3000 ppm (385.7 and 482.7 mg/kg bw/day in males and females respectively) for 91 weeks, revealed a dose-response decrease in body weight in both sexes and, in females, increased cardiac thrombi at 1500 and 3000 ppm. In addition, at 3000 ppm, both sexes showed decreased food consumption, red blood cells, haematocrit and haemoglobin concentration. Female mice also showed decreased brain and kidney weights, a lower percentage of neutrophils and lymphocytes and increased mortality at 3000 ppm. The incidence of unscheduled deaths in mice with cardiac thrombi was statistically significantly different from control groups (US EPA, 2003).

4.1.3 Carcinogenicity and genotoxicity

No information on the carcinogenic or genotoxic potential of atrazine in humans was identified.

In female Sprague-Dawley rats fed atrazine at up to 400 ppm for 2 years effects were noted in tissues influenced by female hormones; these included uterus, ovary, pituitary and mammary gland. Although at 400 ppm no increase in total incidence of mammary tumours was noted, there was a decreased time to onset of mammary tumours. Mammary carcinoma incidence during the first year was 0, 1 and 6 for the control, low and high dose respectively. Overall, it was apparent that there was a treatment-related earlier onset for mammary carcinoma and for combined mammary tumour incidence in the 400 ppm group (CEPA, 2008). A NOEL of 70 ppm was established.

Experimentally, the weight of evidence from a bank of in vitro and in vivo assays indicates that atrazine is not mutagenic (HSDB, 2005

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of atrazine in humans was identified.

In a two-generation study in which atrazine was fed to Charles River (CRCD, VAF/PLUS) rats at dietary levels of up to 500 ppm, decreased body weight, body weight gain and food consumption was noted at the high dose in parent animals of each generation. A significant increase in relative testes weight was also noted in high dose animals of each generation. However, reproductive parameter effects did not appear to show a dose-relationship (US EPA, 2003).

The developmental toxicity of atrazine was assessed in pregnant Charles-River rats orally dosed at up to 700 mg/kg from gestation day 6 to 15 and in pregnant New Zealand White rabbits dosed at up to 75 mg/kg on gestation days 7 to 19. Although dose-related maternal toxicity was evident in both species, no evidence of fetal gross, visceral or skeletal malformations was seen (Infurna et al., 1988).

4.2 Desethyl atrazine

4.2.1 Acute Toxicity

No information on the acute toxic effects of desethylatrazine in humans was identified.

Experimentally, desethylatrazine has moderate acute toxicity in rats with an oral LD50 of 464 mg/kg (Lewis et al., 2007).

4.2.2 Repeat dose toxicity

No information on the effect of repeated exposure to desethylatrazine in humans was identified.

Short-term feeding studies in animals are reported to cause effects similar to those of the parent. Thyroid activation and enlargement of the hormone producing cells of the pituitary of rats were also reported; full details are not publically available (APVMA, 1997).

4.2.3 Reproductive and development toxicity

No information on the repeat dose toxicity of desethylatrazine in humans was identified.

Experimentally, reproductive and developmental toxicity has been assessed in pregnant F344 rats orally dosed with desethylatrazine at up to 0.46 mmol/kg (equivalent to 150 mg atrazine/kg bw) on gestation days 6 to 10. Dams showed significant weight loss at all doses after one or two doses and desethylatrazine was shown to disrupt pregnancy at 0.69 mmol/kg; a 38% loss was reported. In surviving litters, parturition was slightly but significantly delayed at 0.23 and 0.69 mmol/kg. No NOEL was established (Narotsky, 2002).

Searches were made for further, published information on the toxicity of desethylatrazine, and by use of the online programme ChemIDPlus (US NLM, 2003). However, no further information was identified.

Additional information was, therefore, sought through use of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Summaries of the toxicity data predicted for desethylatrazine from these tools are summarised below (Table 1).

Table 1: Predicted toxicity data for desethylatrazine using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$ (range)				
Atrazine (experimental data)	1869	5.8	NOAEL: 9 mg/kg/day (rats)	Negative	Early onset mammary tumours (rats)	Negative
Desethylatra-zine (experimental data)	464	n/d	n/d	n/d	n/d	Delayed parturition (rats and rabbits)
Desethylatra-zine (TOPKAT)	3300 (579.3-10,000)	10,000 (2800-10,000)	MTD (feed/drink) 161.7 mg/kg MTD (oral gavage) 447.7 mg/kg	Negative	Negative	Negative
Desethylatra-zine (DEREK)	n/a	n/a	*Plausible* for hepatotox-icity, respiratory and skin sensitisation in humans	No alert	*Plausible* in humans	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software;

DEREK identified the presence of a number of structural alerts and the following conclusions were drawn:

it is considered plausible (i.e. there is a weight of evidence) that desethylatrazine will be carcinogenic in humans. This endpoint is predicted because desethylatrazine is a Hydrazine.

it is considered plausible (i.e. there is a weight of evidence) that desethylatrazine will cause respiratory sensitisation in humans. This endpoint is predicted because desethylatrazine is a halo-diazine or triazine.

it is considered plausible (i.e. there is a weight of evidence) that desisopropylatrazine will cause skin sensitisation in humans. This endpoint is predicted because desethylatrazine is an activated N-heterocycle.

The analysis by TOPKAT suggested that desethylatrazine was:

non-mutagenic;
non-carcinogenic; and
not a developmental toxicant.

5 Guidelines and Standards

5.1 Atrazine

The EC risk classification is: Xn - Harmful: R48/22; Xi - Irritant: R43; N - Dangerous for the environment: R50, R53. The EC Safety classification is S2, S36/37, S61, S60. Atrazine is classified by WHO as 'unlikely to present acute hazard in normal use' and by the US EPA as 'slightly toxic' (Lewis *et al*., 2007).

An ADI of 0.02 mg/kg bw/day has been established for atrazine using a safety factor (SF) of 100 (Lewis *et al*., 2007); the reference study from which the ADI was derived could not be identified.

5.2 Desethylatrazine

No ADI has been proposed by any authoritative organisation for desethylatrazine. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The experimentally-derived acute oral toxicity of desethylatrazine is somewhat higher than that reported for the parent or predicted by TOPKAT. A robust NOEL for repeat dose toxicity is, however, not available, although predicted oral MTDs range from 161.7 - 447.7 mg/kg depending on form of dosing adopted. Applying the same SF as used for the parent, would suggest a PSDV of 1.6 mg/kg/ bw/day, which is significantly higher than that of 0.02 mg/kg bw/day established for the parent. However, there is convincing experimental as well as predictive toxicity evidence of the reproductive toxic potential of desethylatrazine, a form of toxicity not detected for the parent which does however also appear to have hormonal disrupting potential. Because of this, on a highly precautionary basis, it is proposed to adopt the same ADI as established for the parent compound. This PSDV of 0.02 mg/kg bw/day gives a SF compared the lowest predicted MTD of approx 8000.

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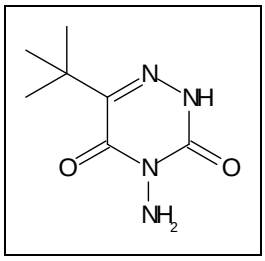
Appendix 12.34 Diketo metribuzin

1 Introduction

Diketo metribuzin (CAS No. 56507-37-0) is a major metabolite of the herbicide metribuzin (4-amino-6-tert-butyl-4,5-dihydro-3-methylthio-1,2,4-triazin-5-one; CAS No. 21087-64-9) in soil. The structure of diketo metribuzin is presented in Figure 1.

Figure 1: Structure of diketo metribuzin



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of metribuzin

Metribuzin is a synthetic organic compound of the triazinone group that is used as a selective herbicide operating via inhibition of photosynthesis. It is often supplied as wettable granules that are mixed with water and used as a spray for the control of grasses and broad-leaved weeds in a range of crops. It is currently sold worldwide under the commercial names Artist and Lexone 2 (USEPA, 2003; Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Metribuzin is highly water soluble (1165 mg/L at pH 5 and 5.53 g/L at pH 9 at 20oC). It is moderately adsorbed on soils. Adsorption decreases with increasing soil pH since metribuzin is adsorbed via a hydrogen-binding mechanism. With measured Koc in the range 3.14 - 81.5 mL/g, metribuzin is highly mobile and readily leached from sandy soils. The primary fate for metribuzin is microbial degradation forming the major metabolites diketo-metribuzin and desamino-diketo-metribuzin (USEPA, 2003; Lewis et al., 2007).

2.2.2 Diketo-metribuzin

Diketo-metribuzin is highly water soluble (1650 mg/L at pH 5 and 5.53 g/L at pH 9, at 20oC). It is moderately persistent in soil under aerobic conditions with a half-life of 29.9 days. A Koc value of 99 ml/g has been reported for diketo-metribuzin suggesting it will have moderate mobility in the environment (Lewis et al., 2007; EFSA, 2006).

2.3 Potential routes of human exposure

Exposure of humans to the parent metribuzin may occur via inhalation, ingestion and dermal contact during occupational handling. Monitoring data indicates that the general population may be exposed to metribuzin via consumption of contaminated foods, and drinking water or inhalation as a result of spraying in nearby agricultural areas (HSDB, 2005).

Although no data on routes of exposure of humans to the metabolite diketo-metribuzin was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of metribuzin, and, to a limited extent, when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of metribuzin in humans.

After a two-day feeding study in which 14[C]-metribuzin was administered to Sprague-Dawley rats, no radioactivity was detected in tissues such as heart, kidney, and liver, suggesting that the metabolites of metribuzin or the parent compound are not accumulated. The presence of the metabolites diketo-metribuzin and deaminated diketo- metribuzin in urine was reported, suggesting that deamination and deketonization are involved in the metabolism of metribuzin. The half-life for elimination of radiolabeled metribuzin was 19.1 - 30.4 hours in male rats and 22.4 - 33.6 hours in female rats (USEPA, 2003; EFSA, 2006).

4 Toxicity Profile

4.1 Metribuzin

4.1.1 Acute Toxicity

No information is available on the acute toxicity of metribuzin in humans.

Metribuzin shows relatively low acute toxicity in rodents; oral LD50s range from 1090 mg/kg to more than 5000 mg/kg in male and female rats, to 245 and 274 mg/kg in male and female Guinea pigs. The dermal LD50 value in both rats and rabbits is > 2000 mg/kg, while the inhalation LC50 is > 2 mg /L in rats and mice. The major effects reflect depression of the CNS (HSDB, 2005; IUPAC Footprint).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of metribuzin in humans was identified.

In a two year feeding study in rats exposed to metribuzin at 35, 100 and 300 ppm, reductions in body weight gain were noted; a NOAEL of 25 ppm (1.3 mg/kg bw/day) was proposed (USEPA 2003).

In a further two year feeding study in rats, metribuzin at 900 ppm (equivalent to 42.2 mg/kg bw/day) affected the bodyweight-relative values for brain, heart, kidney, liver and thyroid. A significant increase in corneal neovascularization was observed in males receiving 300 and 900 ppm of metribuzin (equivalent to 13.8 mg/kg bw/day and 42.2 mg/kg bw/day respectively). Changes in thyroid hormone levels (increased T4 and decreased T3) were noted at the low dose (30 ppm; 1.3 mg/kg bw/d) as well as at higher doses. Therefore a NOAEL could not be established for this study (USEPA, 2003).

In a 90 day feeding study in dogs, metribuzin at 1.25, 3.75 and 12.5 mg/kg bw/day showed no sign of toxicity (EPA 2003). Dermal application of 1000 mg/kg bw/day (as 70% wettable powder) for three weeks caused no observable effects in rats and, in a three week inhalation study with rats (aerosol exposure six hours per day, five days a week), an atmospheric concentration of 31 mg/L was also without observable effects (HSDB, 2005).

4.1.3 Carcinogenicity and mutagenicity

No information on the carcinogenic or mutagenic potential of metribuzin in humans was identified.

In the two year feeding study in rats exposed to metribuzin at 35, 100 and 300 ppm, no increase in incidence of neoplastic changes were reported (USEPA 2003).

Female rats administered metribuzin at 300 and 900 ppm (equivalent to 13.8 and 42.2 mg/kg bw/day) via the diet showed developed ovarian cysts. Changes in thyroid hormone levels (increased T4 and decreased T3) were also noted, however as these were at both low and high doses a NOAEL could not be established (USEPA, 2003).

Tests for mutagenicity were negative. However, in one study, S-9 activated metribuzin was clastogenic to CHO cells and subsequent investigations indicated metribuzin exposure resulted in adduct formation (USEPA, 2003).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of metribuzin in humans was identified.

In a three-generation study in rats, metribuzin at 1.75, 5, or 15 mg/kg bw/day did not influence reproduction or development (USEPA, 2003).

In a developmental toxicity study in rabbits administered metribuzin in the diet at 10, 30, 85 mg/kg bw/day, reduced body weight and food consumption and maternal toxicity were noted at 30 mg/kg bw/day and above; the maternal NOAEL was 10 mg/kg bw/day.

In a further study which administered metribuzin at 15, 45, and 135 mg/kg bw/day in the diet, maternal toxicity was observed at 45 mg/kg bw/day; no embryotoxic or teratogenic effects were observed. Based on these studies, an overall NOAEL for reproduction and developmental toxicity of > 135 mg/kg bw/day was established (USEPA, 2003).

4.2 Diketo-metriburin

No information on the repeat dose toxicity of diketo-metribuzin in humans was identified.

Searches were made for publicly available information on the toxicity of diketo-metribuzin. However, no information has been identified.

Additional information was, therefore, sought through use of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK identified the presence of several structural alerts and the following conclusions were drawn:

it is considered plausible (i.e. there is a weight of evidence) that diketo-metribuzin will be carcinogenic in humans. This endpoint is predicted because diketo-metribuzin is a hydrazine.

it is considered plausible (i.e. there is a weight of evidence) that diketo-metribuzin will cause hepatotoxicity in humans. This endpoint is predicted because diketo-metribuzin is a hydrazine.
it is considered plausible (i.e. there is a weight of evidence) that diketo-metribuzin will cause skin sensitisation in humans. This endpoint is predicted because diketo-metribuzin is a hydrazine.

- it is considered plausible (i.e. there is a weight of evidence) that diketo-metribuzin will cause teratogenicity in humans. This endpoint is predicted because diketo-metribuzin is a hydrazine.

The analysis by TOPKAT suggested that diketo-metribuzin was:

- non-carcinogenic;
- non-mutagenic

Prediction of the reproductive and developmental toxicity of diketo-metribuzin using TOPKAT was not possible.

Table 1: Predicted toxicity data for diketo-metribuzin using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Metribuzin (experimental data)	1090 (rats)	>648	NOAEL 1.3 mg/kg/d Main target organs: liver and thyroid	Some evidence of genotoxicity	Non-carcinogen	NOAEL Maternal: 10 mg/kg/d Reproduction and development: > 135 mg/kg/d
Diketo-metribuzin (TOPKAT)	5500 (920-10000)	10000 (10000-10000)	MTD (feed/drink) Unreliable estimate MTD (oral gavage) Unreliable estimate	Non-mutagen	UE	UE
Diketo-metribuzin (DEREK)	n/a	n/a	*Plausible* for hepatotox-icity in humans	*OPEN* for mutagenicity	*Plausible* for carcinogenicity in humans	*Plausible* for teratogenicity in humans

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software; UE – unreliable estimate

5 Guidelines and Standards

5.1 Metribuzin

Metribuzin is classified by WHO as 'moderately hazardous', and by the US EPA as III (Caution-slightly toxic). The EC risk classification is: Xn - Harmful: R22; N - Dangerous for the environment: R50, R53 (Lewis *et al*., 2007).

An ADI of 0.013 mg/kg bw/day has been established for metribuzin based on a two year rat study with a safety factor (SF) of 100 applied. In this study the NOAEL was 1.3 mg/kg bw/day. This ADI did not take account of another study showing that the NOAEL was < 1.3 mg/kg bw/day, based on the influence of metribuzin at 1.3 mg/kg bw/day on levels of thyroid hormones.

5.2 Diketo-metribuzin

Due to the lack of experimental data, it has not been possible to establish a robust NOAEL for diketo-metribuzin, and no ADI has been proposed by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

No oral MTD could be predicted using TOPKAT for diketo-metribuzin. Acute toxicity of the metabolite is predicted to be less than that of the parent. In the absence of any repeat dose toxicity data it is proposed to adopt the same ADI as established for the parent compound. However, it should be noted that given the number of structural alerts for diketo-metribuzin, determination of a specific ADI should be given priority.

6 References

EFSA (2006) Conclusion Regarding the Peer Review of the Pesticide Risk Assessment of the Active Substance: Metribuzin. Available at: http://www.efsa.europa.eu/cs/BlobServer/PRAPER_Conclusion/praper_concl_sr88_metribuzin_en.pdf

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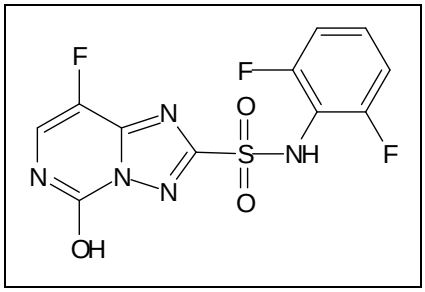
Appendix 12.35 N-(2,6-difluorophenyl)-8-fluoro-5-hydroxy[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide

1 Introduction

N-(2,6-difluorophenyl)-8-fluoro-5-hydroxy[1,2,4]triazolo[1,5-c]pyrimidine-2-sulfonamide (5-hydroxy-XDE-570) is a major metabolite of the herbicide florasulam (CAS No. 145701-23-1) in soil. The chemical structure of XDE -570 is presented in Figure 1.

Figure 1: Chemical structure of 5-hydroxy-XDE-570



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of florasulam

Florasulam is a selective herbicide belonging to the chemical group of triazolopyrimidine. It is absorbed by roots and shoots and inhibits the plant enzyme acetolactate synthase in susceptible species. Often supplied as wettable granules or soluble concentrate which are mixed with water and applied as a spray, florasulam is sold worldwide for use on cereal grains including wheat, oats, rye and triticale at a maximum rate of 0.00446 pounds ai/acre. A number of products contain florasulam, such as Barton WG and Boxer (USEPA, 2007).

2.2 Environmental fate

2.2.1 Parent

Florasulam is highly water soluble (6360 mg/L at 20oC). The sorption Koc for florasulam was measured in the range of 4 - 54 mL/g, indicating that the chemical is very mobile in soil. The compound is rapidly broken down in soil with a typical half-life of 8.5 days under field conditions (IUPAC Footprint) with 5-hydroxy-XDE-570 being the major degradate (USEPA, 2007).

2.2.2 Metabolite: 5-hydroxy-XDE-570

In soil, 5-hydroxy-XDE-570 is slowly degraded with a half-life of 10 to 56 days in aerobic soil. It is more mobile than the parent compound. It has a greater potential to leach to groundwater due to its slower biotransformation in aerobic soil and higher water solubility. (Pest Management Regulatory Agency, 2001; USEPA 2007).

2.3 Potential routes of human exposure

The exposure of humans to the parent florasulam may occur through occupational handling (inhalation and dermal routes). Exposure to both parent and its degradate 5-hydroxy-XDE-570 may also occur through ingestion of contaminated water and food.

Although no data on routes of exposure of humans to the metabolite 5-hydroxy-XDE-570 was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of florasulam, and, to a limited extent, when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of florasulam in humans.

In rats, radiolabeled florasulam has been shown to be readily absorbed, with a rapid elimination, mostly within 12 hour, via urine. Total radioactivity in the urine was approximately 90 - 92% of the dose following single or repeated low-dose treatment, and 81-85% of the dose of 500 mg/kg bw. Radioactivity in the faeces accounted for 5 - 7% at 10 mg/kg and 14 % at 500 mg/kg (USEPA, 2007; full study details not available).

No information on the toxicokinetics of 5-hydroxy-XDE-570 was identified.

4 Toxicity Profile

4.1 Florasulam

4.1.1 Acute Toxicity

No information is available on the acute toxicity of florasulam to humans.

Florasulam has low or minimal acute toxicity via oral, dermal and inhalation routes. In rats and mice, the oral LD50 is > 5000 mg/kg bw. The dermal LD50 in rabbits is > 2000 mg/kg bw and the inhalation LC50 in rats is >5.0 mg/L. (USEPA, 2007).

Florasulam is non-irritating to the eye and skin and is not a skin sensitizer (USEPA, 2007).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of florasulam in humans was identified.

In rats fed diets containing florasulam at levels intended to achieve intakes of 250 or 500 mg/kg bw/day, slight nephrotoxicity (increased kidney weight, hypertrophy and slight multi-focal mineralization of papilla) were noted in males. Papillary necrosis and hyperplasia of the transitional epithelium (papilla) were also observed in males given at 500 mg/kg bw/day. In female rats, given at 500 mg/kg bw/day a decrease in body weight gain was noted. A NOEAL of 10 mg/kg bw/day was proposed (USEPA, 2007; full study details unavailable).

Elevated blood alkaline phosphatase activity, a marker of liver toxicity, was noted in dogs 90 days into a 1 year study in which florasulam was administered via the diet at 50 mg/kg bw/day. After 1 year, although alkaline phosphatase activity was still elevated, no changes in liver weight or pathology were noted. Decreased body weight and food consumption and adrenal zona reticularis and fasciculate vaculation were also apparent in treated animals at this time. The NOEAL was determined as 5 mg/kg bw/day (USEPA, 2007; full study details unavailable).

4.1.3 Carcinogenicity and mutagenicity

No information on the carcinogenic or mutagenic potential of florasulam in humans was identified.

In a combined chronic toxicity and carcinogenicity study, rats were exposed via the diet to florasulam at 0, 250, 500 mg/kg bw/day. No effects on tumour incidence was observed. Based on slight nephrotoxicity, a LOAEL of 250 mg/kg bw/day was established (USEPA, 2007; full study details unavailable).

Florasulam is negative in mutation and chromosomal aberration assays (USEPA, 2007; full study details unavailable).

4.1.4 Reproductive and development toxicity

No information on reproductive or developmental toxicity of florasulam in humans was identified.

In a developmental toxicity study in rabbits, florasulam at 750 mg/kg bw/day caused decreased body weight and food consumption and increased kidney weight in dams. The maternal NOEAL was 250 mg/kg bw/d but the developmental NOEAL was 750 mg/kg bw/d (USEPA, 2007; full study details unavailable).

4.2 5-Hydroxy-XDE-570

No information on the repeat dose toxicity of 5-hydroxy-XDE-570 in humans was identified.

Searches were made for published information on the toxicity of 5-hydroxy-XDE-570, including use of the online programme ChemIDPlus (http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp). However, no data were identified.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK identified a number of structural alerts in the molecule and the following conclusions were drawn:

- it is considered plausible (i.e. there is a weight of evidence) that 5-hydroxy-XDE-570 will cause methaemoglobinaemia in humans. This endpoint is predicted because 5-hydroxy-XDE-570 is an aniline precursor.

- it is considered plausible (i.e. there is a weight of evidence) that 5-hydroxy-XDE-570 will be carcinogenic in humans. This endpoint is predicted because 5-hydroxy-XDE-570 is a purine.

- it is considered plausible (i.e. there is a weight of evidence) that 5-hydroxy-XDE-570 will cause ocular toxicity in humans. This endpoint is predicted because 5-hydroxy-XDE-570 is an aryl sulphonamide

The analysis by TOPKAT also suggested that 5-hydroxy-XDE-570 was:

- non-carcinogenic

Predictions of the mutagenic potential and reproductive and developmental toxicity for 5-hydroxy-XDE-570 using TOPKAT was not possible.

Table 1 Predicted toxicity data for 5-hydroxy-XDE-570 using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$ (range)				
Florasulam (experimental data)	>5000	>5	NOAEL (1 year study in dogs) 5 mg/kg bw/d	Negative	Negative	NOAEL Paternal toxicity: 250 mg/kg bw/d Development: 750 mg/kg bw/d
5-hydroxy-XDE-570 (TOPKAT)	102.9 (15.7-676.9)	10,000 (10,000-10,000)	MTD (feed/drink) UE MTD (oral gavage) UE	UE	Negative	Negative
5-hydroxy-XDE-570 (DEREK)	n/a	n/a	*Plausible* for methaemoglobinaemia and ocular toxicity in humans	No alert	*Plausible* in humans	No alert

LD_{50} : lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; - : no data; UE – unreliable estimate; MTD – maximum tolerated dose; n/a – prediction not applicable to software.

5 Guidelines and Standards

5.1 Florasulam

The EC risk classification is: N - Dangerous for the environment: R50, R53. Florasulam is categorised by WHO as 'product unlikely to present acute hazard in normal use' (Lewis *et al*., 2007).

An ADI of 0.05 mg/kg bw/day has been established for florasulam based on a 1 year chronic toxicity study in dogs which gave a NOAEL of 5 mg/kg bw/d; a safety factor (SF) of 100 was applied (Lewis *et al*., 2007).

5.2 5-Hydroxy-XDE-570

Due to the lack of experimental data, it has been not possible to establish a robust NOAEL for 5-hydroxy-XDE-570, and no ADI has been proposed by any authoritative organization.

DEREK and TOPKAT were unable to estimate a MTD for this metabolite under conditions of repeat exposure, and identified a number of possible (but uncertain) activities, some of which are not possessed by the parent. TOPKAT has predicted a much lower oral $LD_{50\ than}$ for florasulam, although this does not appear to be the case via the inhalation route.

For the purposes of this risk assessment, it is proposed – on a highly precautionary basis- to adopt the parental ADI of 0.05 mg/kg bw/day. However, it should be noted that, on the basis of the predicted toxicity profile, it is possible that the ADI for

florasulam may provide an inadequate margin of safety for 5-hydroxy-XDE-570. Furthermore, 5-hydroxy-XDE-570 is more persistent than the parent compound in soil, and has the potential to leach to groundwater. Therefore, human exposure to 5-hydroxy-XDE-570 may be a higher risk than florasulam.

6 References

Pest Management Regulatory Agency (2001) Florasulam EF-1343 Suspension Concentrate Herbicide. Available at: http://dsp-psd.pwgsc.gc.ca/Collection/H113-7-2001-12E.pdf

USEPA (2007) Florasulam Chemical Documents. Available at: http://www.epa.gov/opprd001/factsheets/florasulam.htm

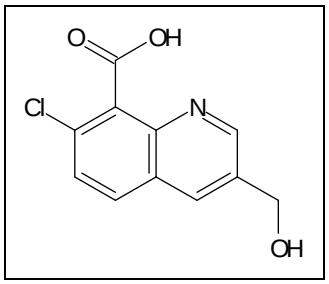
Appendix 12.36 BH518-4

1 Introduction

BH518-4 is a metabolite of the herbicide quinmerac (CAS No. 90717-03-6).

The structure of BH518-4 is presented in Figure 1.

Figure 1: Structure of BH518-4



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of quinmerac

Quinmerac is a new herbicidally active ingredient belonging to the chemical group of quinoline carboxylic acids. Quinmerac, used in formulated mixtures with either chloridazon or metazachlor, offers flexible and potent possibilities for broad spectrum weed control. It is used to control broad-leaved weeds on a range of crops including cereals, rape and beet. Quinmerac is taken up via the root as well as the leaves and is systemic within the plant. The direction of translocation is preferably basipetal. Leaf uptake of quinmerac and its combination partners is enhanced when applied with adjuvants. Quinmerac itself does not influence photosynthesis directly. It enhances, however, the inhibition of photosynthesis by chloridazon synergistically. Quinmerac may stimulate the production of ethylene through its auxin activity in susceptible species. (Lewis et al., 2007; Walter et al, 1994)

It is sold worldwide under the trade names including Oryx, Katamaran and Boomerang.

2.2 Environmental fate

2.2.1 Parent

Quinmerac is highly water soluble (107,000 mg/L at 20oC). In soil, the compound is degraded with a typical half life of 24 days under field conditions. The sorption Koc is measured in the range of 19.2-184.8 mL/g and is sensitive to PH (increases with decreasing pH), suggesting that quinmerac is moderately mobile (Lewis et al., 2007).

2.2.2 Metabolite: BH518-4

No information on the physiochemical properties or environmental fate of BH518-4 was identified.

2.3 Potential routes of human exposure

The potential exposure of humans to the parent quinmerac may occur through ingestion of contaminated food and water (oral route), or through occupational handling or bystander exposure (inhalation or dermal contact).

Although no data on routes of exposure of humans to the metabolite BH518-4was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of quinmerac, or when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of quinmerac or BH518-2 in humans. No experimental information on the toxicokinetics of quinmerac or BH518-2 was identified.

4 Toxicity Profile

4.1 Quinmerac

4.1.1 Acute toxicity

No information is available on the acute toxicity of quinmerac to humans.

In rats, quinmerac showed low toxicity, with LD50 >4070 mg/kg bw and LC50 >5.7 mg/L when given by the oral or inhalation routes. The dermal toxicity of quinmerac was tested in rabbits with LD50 >2000 mg/kg bw. Quinmerac was not irritating to the eye. It may cause sensitization to the skin (BASF, 2009).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of quinmerac in humans, or experimental data was identified.

4.2 BH518-4

No information on the repeat dose toxicity of BH518-4 in humans was identified. Searches were made for publicly available information on the toxicity of BH518-4 and by use of the online programme ChemIDPlus (http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp). However, no information relating to BH518-4 toxicity was identified.

Additional information was, therefore, sought through application of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Summaries of the toxicity data predicted for BH518-4 from these tools are summarised below (Table 1).

Table 1: Predicted toxicity data for BH518-4 using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity/ Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Quinmerac (experimental data)	>5000	>5.7	n/d	n/d	n/d	n/d
BH518-4 (TOPKAT)	10,000 (1700 - 10,000)	10,000 (3000-10,000)	MTD (feed/drink): 78.8 mg/kg MTD (oral gavage): 218. 3 mg/kg	Negative	Negative	Indeterminate
BH518-4 (DEREK)	n/a	n/a	No alert	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software

DEREK did not identify any structural alerts in the BH518-4 molecule.

The analysis by TOPKAT suggested that BH518-4 was:

non-carcinogenic;
non-mutagenic

Prediction of reproductive and developmental toxicity of BH518-4 using TOPKAT was not possible.

5 Guidelines and Standards

5.1 Quinmerac

EC risk classification for quinmerac is; N - Dangerous for the environment: R50, R53. EC safety classification is S60, S61. Quinmerac is classified by WHO as 'unlikely to present acute hazard in normal use' (Lewis *et al*., 2007).

An ADI of 0.079 mg/kg bw/day has been established for quinmerac (Lewis *et al*., 2007). This was based on a dog study with safety factor of 100; however the reference of the study could not be identified.

5.2 BH518-4

Due to the lack of experimental data, no ADI has been proposed by any authoritative organisation for BH518-4. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The highest oral MTD predicted by TOPKAT for BH518-4 is 218.3 mg/kg bw/day. Applying a SF of 100 would give a nominal value of 2.18 mg/kg bw/day, which would be significantly above the established ADI of 0.079 mg/kg bw/day for the parent quinmerac. Given this, it is proposed to adopt a PSDV of 0.079 mg/kg bw/d for

BH518-4, i.e. the same value as for the parent; this will provide an overall SF of >2000.

6 References

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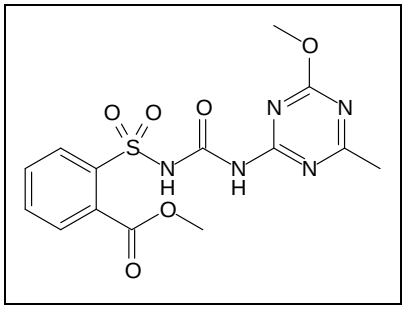
Appendix 12.37 Metsulfuron-methyl (methyl 2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoyl-sulfamoyl)-benzoate

1 Introduction

Metsulfuron-methyl (CAS No. 74223-64-6) is the BSI name for methyl 2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)benzoate. It is a sulfonylurea herbicide. Metsulfuron-methyl is also the predominant metabolite in soil and drinking water of iodosulfuron-methyl-sodium which belongs to the general class of herbicides termed sulfonylureas.

The structure of metsulfuron-methyl is presented in Figure 1.

Figure 1: Structure of metsulfuron-methyl



2 Use and environmental fate and potential human exposure routes

2.1 Use of metsulfuron-methyl

Metsulfuron-methyl is used to control grasses and broad-leaved weeds mainly in cereals and land temporarily removed from production. As a systemic compound with foliar and soil activity, metsulfuron-methyl inhibits plant amino acid synthesis and therefore cell division in the shoots and roots of the plant. It is biologically active at low use rates and works rapidly after it is taken up by the plant.

Metsulfuron-methyl is sold worldwide often under the product name Ally. It is often supplied as wettable granules and mixed with water to form a spray. The maximum application rate for metsulfuron-methyl is 6 g a.i/ha (PSD, 1995).

2.2 Environmental fate

2.2.1 Parent

Iodosulfuron-methyl-sodium is highly water soluble (25000 mg/L at 20oC). In the soil, the compound is degraded with a typical half-life of 8 days under field conditions. The major metabolites (fraction >10% of applied rate) are AE F059411, AE F161778 and metsulfuron-methyl. The sorption Koc for iodosulfuron-methyl-sodium was measured in the range of 10 – 152 mL/g, indicating very high to high mobility (EC, 2000).

2.2.2 Metabolite: Metsulfuron-methyl

Metsulfuron-methyl is highly water soluble (2790 mg/l at 20oC). In soil the compound is broken down both by chemical hydrolysis and by microbial degradation. The typical half-life is 10 days under field conditions with breakdown being more rapid at lower soil pH, higher temperatures, and higher levels of soil moisture. The major metabolites (fraction >10% of applied rate) are methyl 2-(aminosulfonyl)benzoate (Ref: IN-D5803), 2-(aminosulfonyl) benzoic acid (Ref: IN-D5119), phenylurea (Ref: IN-B5685), and saccharin (Ref: IN-00581). The sorption Koc for metsulfuron-methyl was measured in the range of 4 – 60 ml/g, indicating very high mobility (EC, 2000).

2.3 Potential routes of human exposure
Exposure of humans to metsulfuron-methyl may occur through ingestion of contaminated food and water (oral route), or through occupational handling or bystander exposure (inhalation or dermal contact).

3 Toxicokinetics
No information on the toxicokinetics of metsulfuron-methyl in humans is available.

Total recovery of radiolabelled metsulfuron-methyl administered to rats through the oral route was 91.6-103.8 %. The primary route of excretion is via the urine which accounted for approx. 71 - 95% with faecal elimination accounting for 4.8 - 13.3%. Excretion was almost complete within 48 hours. Elimination half-lives (males and females) were estimated to be 13-16 hours for single low dose group, 9-12 hours for 21-day dietary exposure group, and 23-29 hours for single high dose group. Tissue burdens were generally minimal (< 0.1% to 1%). The parent compound was recovered in both urine and faeces of all treatment groups and accounted for most of the urinary and faecal radioactivity (77 - 90% and 1.8 - 6.2% of administered dose, respectively). Four metabolites (saccharin and three other metabolites (I, II and III)) were found in both matrices and accounted for approximately 5.4 - 8.2% of administered dose. Two of the metabolites appeared to result from the sequential hydrolysis reactions terminating in the formation of saccharin while one was formed by cleavage of the two-ring structures. The metabolite profiles were qualitatively similar in urine and faeces for parent compound and the four metabolites (USEPA, 2002; full study details not available).

4 Toxicity Profile
4.1 Metsulfuron-methyl
4.1.1 Acute Toxicity
No information on human acute toxicity has been identified.

In rats, metsulfuron-methyl has low acute toxicity; LD50 s are > 5000 mg/kg bw and LD50 > 2000 mg/kg bw for the oral and dermal routes while the inhalation LC50 is > 5 mg/L (Lewis et al., 2007).

It is not irritant to the eyes of rabbits and does not cause skin sensitisation in Guinea pigs (EC, 2000).

4.1.2 Repeat dose toxicity
No information on the repeat dose toxicity of metsulfuron-methyl in humans was identified.

In a 90 day study, rats were orally dosed with metsulfuron-methyl. Based on transient decrease in body weight gain, NOAELs were 68 mg/kg bw/day and 64 mg/kg bw/day for males and females, respectively, and LOAELs were 521 and 659 mg/kg bw/day for male and female rats, respectively(USEPA, 2002; full study details not available).

In a dermal study in rabbits, a NOAEL of 12 mg/kg bw/day and LOAEL of 500 mg/kg bw/day were established based on skin lesions characterized by diffuse/multifocal dermatitis. The systemic NOAEL was higher at 125 mg/kg/day although the systemic LOAEL was the same (500 mg/kg/day); this was based on an increased incidence of diarrhoea (USEPA, 2002; full study details not available).

4.1.3 Carcinogenicity and mutagenicity

No information was found on the carcinogenic and mutagenic potential of metsulfuron-methyl in humans.

In a carcinogenicity study in rats, metsulfuron-methyl at 25 mg/kg bw/day caused no effect while 250 mg/mg bw/day caused body weight gain reduction. Based on this, a LOAEL was established as 250 mg/mg bw/day for males and females. No evidence of carcinogenicity was found (USEPA, 2002; full study details not available).

All mutagenicity tests produced negative results (USEPA, 2002; full study details not available).

4.1.4 Reproductive and development toxicity

In a two-generation study in rats, NOAELs for systemic parental toxicity of 34 mg/kg bw/day for males and 43 mg/kg bw/day for females were proposed based on decreased pre-mating body weight gains by F0 males and females at higher dose. The NOAELs for reproductive and developmental toxicity were 342 mg/kg bw/day for males and 475 mg/kg bw/day (USEPA, 2002; full study details not available).

In rabbits, parental toxicity NOAEL was 25 mg/kg bw/day while LOAEL was 1,000 mg/kg bw/day based on increased mortality, decreased body weight gains, and clinical signs of anorexia, red or orange urine and /or exudate. Developmental NOAEL was 700 mg/kg bw/day. No developmental LOAEL was established (EC, 2000; USEPA, 2002 - full study details not available).

A summary of the toxicity data for metsulfuron-methyl is presented below (Table 1).

Table 1: Experimental toxicity data for metsulfuron-methyl

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Metsulfuron methyl (experimental data)	>5000	>5	NOAEL 64 mg/kg bw/d Main effect: Transient reduction in body weight gain	Negative	Negative	NOAEL Reproduction and Development ≥ 342 mg/kg bw/d

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested.

5 Guidelines and Standards

5.1 Metsulfuron-methyl

The EC risk classification is: N - Dangerous for the environment: R50, R53. WHO classified metsulfuron-methyl as 'unlikely to present acute hazard in normal use'. Metsulfuron-methyl is classified by EPA as Category III, and must bear the signal word "Caution" on commercial products (Lewis *et al.,* 2007).

An ADI of 0.25 mg/kg bw/day has been established for metsulfuron-methyl based on a carcinogenicity study in rats in which NOAEL was 25 mg/kg bw/day, with a safety factor (SF) of 100 applied.

6 References

EC (2000) Review report for the active substance metsulfuron-methyl. 7593/VI/97-final. Available at: http://ec.europa.eu/food/plant/protection/evaluation/existactive/list1-13_en.pdf

Lewis K, Green A, Tzilivakis J (2007) Pesticide database holding fate and ecotoxicological values. Report DL24 of the FP6 EU-funded FOOTPRINT project. Available at http://www.eu-footprint.org

PSD (Pesticides Safety Directorate) (1995) Evaluation on: Review of the Environmental Persistence of Metsulfuron-Methyl and Thipensulfuron-Methyl. Available at: http://www.pesticides.gov.uk/PSD_PDFs/Evaluations/119_environmental_persistence_of_metsulfuron-methyl_and_thifensulfuron-methyl.pdf

USEPA (2002) Metsulfuron Methyl; Pesticide Tolerance. Federal Register, 67(152), 51088-51097. Available at: http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=2002_register&docid=02-19807-filed.pdf

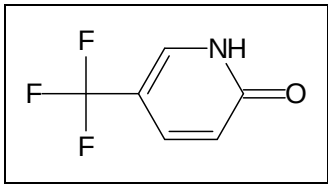
Appendix 12.38 5-trifluoromethyl-pyrid-2-one

1 Introduction

5-Trifluoromethyl-pyrid-2-one is a metabolite of the herbicide fluazifop-P-butyl (butyl (2*R*)-2-[4-[[5-(trifluoromethyl)-2-pyridinyl]oxy]phenoxy]propanoate; CAS No. 79241-46-6).

The structure of 5-trifluoromethyl-pyrid-2-one, is presented in Figure 1.

Figure 1: Structure of 5-trifluoromethyl-pyrid-2-one



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Fluazifop-P-butyl

Fluazifop-P-butyl is a selective post-emergence phenoxy herbicide used for control of most annual and perennial grass weeds in cotton, soybeans, stone fruits, asparagus, coffee, and others (EXTOXNET, 1996).

The herbicide is sold worldwide under the tradenames, Fusilade Max and Greencrop Bantry and is often supplied as an emulsion concentrate which is mixed with water and applied as a spray (Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Fluazifop-P-butyl has low solubility in water (0.93 mg/L) and, in assessing the sorption to soil, a Koc value of 5836 L kg-1 has been described, suggesting no mobility in the environment (Lewis et al., 2007).

Fluazifop-P-butyl is susceptible to degradation in soil under aerobic conditions, with a degradation half-life of 6 an 8.2 days under laboratory (20oC) and field conditions respectively; fluazifop-P-butyl is not considered to be persistent (Lewis et al., 2007).

2.2.2 Metabolite: 5-trifluoromethyl-pyrid-2-one

5-trifluoromethyl-pyrid-2-one is one of the metabolites formed through metabolism and environmental degradation of fluazifop-P-butyl.

No information relating to either the physicochemical properties or environmental fate of the metabolite 5-trifluoromethyl-pyrid-2-one was identified.

2.3 Potential routes of human exposure

Occupational exposure to fluazifop-P-butyl may occur through inhalation and dermal contact with this herbicide during or after its application or at workplaces where fluazifop-P-butyl is produced or used. The general population may be exposed to fluazifop-P-butyl via dermal contact, inhalation of ambient air or through ingestion of drinking water and food contaminated with the herbicide (HSDB, 2007).

Although no data on routes of exposure of humans to the metabolite 5-trifluoromethyl-pyrid-2-one was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of fluazifop-P-butyl,

and, to a limited extent, when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics of parent and metabolite

Toxicokinetics of fluazifop-P-butyl has been assessed in three male volunteers. Following a single dose of 0.07 mg, fluazifop-P-butyl was rapidly absorbed, with plasma concentrations reaching a peak within one to two hours; the estimated half-life was 9 – 37 hours. Excretion via urine accounted for 80 – 93% of administered dose over a period of six days (PSD, 1988).

In rats given a single oral dose of 1 mg of 14[C]-fluazifop-P-butyl, absorption was rapid with distribution to many tissues. Highest levels of radiolabel were found in blood, fat, kidneys and liver of males and fat of females. Metabolism was extensive, with little unchanged fluazifop-P-butyl excreted in urine, and involved hydrolysis of the esters to free acid followed by inversion of the S-enantiomer to the R-enantiomer. The major urinary metabolite (95% of urinary excretion) was free fluazifop acid which was also present in faeces. Major biliary excretion products included fluazifop butyl and its taurine conjugate; their relative predominance was sex-related. Excretion was mainly via urine (around 90% within 24 hours in females). In males, urinary excretion accounted for 37 - 50% and faecal excretion 47 - 57% over a 10 day period (PSD, 1988).

No information relating to the toxicokinetics of 5-trifluoromethyl-pyrid-2-one was identified.

4 Toxicity Profile

4.1 Fuazifop-P-butyl

4.1.1 Acute Toxicity

No information on the acute effects of fluazifop-P-butyl in humans was found.

Experimentally, fluazifop-P-butyl is moderately toxic in rats; LD50's are 2000 mg/kg, 2100 mg/kg and 5.24 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007).

Fluazifop-P-butyl is a mild irritant to the skin and eye. No evidence of skin sensitization has been found (Kidd & James, 1991).

4.1.2 Repeat dose toxicity

No information on the effects of repeated exposure of fluazifop-P-butyl to humans was found.

Experimentally, in a two-year feeding study in Sprague-Dawley rats fed fluazifop-P-butyl in the diet at levels adjusted to achieve up to 3.0 mg/kg bw/day, no effects were noted on survival body weight gain or food consumption. Some sporadic differences in haematological and clinical chemistry parameters were noted but were not consistent. After one year of treatment, absolute relative kidney and adrenal weights were increased in high dose males and decreased absolute testes weight; bodyweight relative testes weights were noted at 1 or 2 mg/kg bw/day. A NOEL of 0.3 mg/kg bw/day was established, based on reduction in testes weight (PSD, 1988).

4.1.3 Carcinogenicity and genotoxicity

No information on the carcinogenic or genotoxic potential of Fluazifop-P-butyl in humans was identified.

In the two-year feeding study in rats described above, no evidence of carcinogenicity was noted (PSD, 1988).

Fluazifop-P-butyl was not mutagenic in the Ames test with or without metabolic activation and is not considered mutagenic (PSD, 1988).

4.1.4 Reproductive and development toxicity
No information was found on the reproductive and developmental effects of fluazifop-P-butyl in humans.

In a two-generation reproductive toxicity study, Sprague-Dawley rats were fed diets containing fluazifop-P-butyl at up to 250 ppm. An increase in absolute and bodyweight-relative liver weights was noted in parental animals given 250 ppm and an increase in absolute and relative kidney weights in both parents and weanlings with associated increase in geriatric nephropathy and nephrocalcinosis. No adverse effect on male reproductive performance was noted although absolute and bodyweight-relative testes weight were consistently reduced in parental males given 80 or 250 ppm and, in some instances at 10 ppm; an associated tubular degeneration was also noted in some instances. However, in females, reproductive effects included prolonged gestation at 80 and 250 ppm, and decreased litter size, reduced birth weight and body weight gain at 250 ppm. Developmental effects included reduced fetal weights in all treated groups, and increased hydroureter and hydronephrosis, enlarged anterior fontanelles and changes in and ocular effects (including microphthalmia) at 250 ppm. A dose-related effect on ossification including some changes that extended to the 10 ppm group (PSD, 1988).

4.2 5-trifluoromethyl-pyrid-2-one
4.2.1 Acute Toxicity
No information on the acute effects of 5-trifluoromethyl-pyrid-2-one in humans or animals was found.

4.2.2 Repeat dose toxicity
No information on the effects of repeated exposure to 5-trifluoromethyl-pyrid-2-one in humans or animals was found.

Searches were made for published information on the toxicity of 5-trifluoromethyl-pyrid-2-one, including use of the online programme ChemIDPlus (US NLM, 2003). However, No additional information was identified.

Additional information was, therefore, sought through use of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK did not identify any structural alerts in the molecule.

The analysis by TOPKAT suggested that 5-trifluoromethyl-pyrid-2-one was:

- carcinogenic; and
- mutagenic; and
- a developmental toxicant

Table 1 Predicted toxicity data for 5-trifluoromethyl-pyrid-2-one using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Fulizafop-P-butyl (experimental data)	2000	5.24	NOAEL: 0.3 mg/kg/day (rats)	Negative	Negative	Negative
5-trifluoro-methyl-pyrid-2-one (TOPKAT)	14.3 (2.5 – 82.9)	UE	MTD (feed/drink) 0.514 mg/kg MTD (oral gavage) 0.196 mg/kg	Mutagenic	Positive	Developmental toxicant
5-trifluoro-methyl-pyrid-2-one (DEREK)	n/a	n/a	No alert	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software; UE – unreliable estimate

5 Guidelines and Standards

5.1 Fulizafop-P-butyl

The EC risk classification is: Reproduction risk category 3: R63; N - Dangerous for the environment: R50, R53. The EC Safety classification is S2, S29, S36/37, S46, S60, S61. Fulizafop-P-butyl is classified by WHO as 'slightly hazardous' and by the US EPA as 'slightly toxic' (Lewis *et al*., 2007).

An ADI of 0.01 mg/kg bw/day has been established for fulizafop-P-butyl, based on a two-year rat feeding study, using a safety factor (SF) of 100 (Lewis *et al*., 2007). Although adopted in the risk assessment, full study details on which this was based were not identified.

5.2 5-trifluoromethyl-pyrid-2-one

No ADI has been proposed by any authoritative organisation for 5-trifluoromethyl-pyrid-2-one. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

Overall, the two predictive systems employed gave markedly different predictions as to the mutagenic, carcinogenic and developmental toxicity of the metabolite. While there is some evidence that the parent may have some reproductive and developmental activity, fulizafop-P-butyl does not appear to be either mutagenic or carcinogenic. Despite this, the tolerance to repeated exposure of 5-trifluoromethyl-pyrid-2-one is predicted to be similar to that of the parent. The highest oral MTD

predicted by TOPKAT for 5-trifluoromethyl-pyrid-2-one is 0.514 mg/kg when given via feed/water. Applying a SF of 100 would give a nominal value of 0.0051 mg/kg bw/day which is similar to the established ADI of 0.01 mg/kg bw/day for the parent fulizafop-P-butyl. It is therefore proposed to adopt a PSDV of 0.01 mg/kg bw/d for 5-trifluoromethyl-pyrid-2-one, i.e. the same value as for the parent. The heightened uncertainty with regard to the possible additional toxic activities of the metabolite should, however, be noted in the risk assessment.

6 References

Extension Toxicology Network (EXTOXNET, 1996) Pesticide Information Profiles: Fluazifop-p-butyl. Available at: http://extoxnet.orst.edu/pips/fluazifo.htm

Kidd H & James DR., Eds. The Agrochemicals Handbook, Third Edition. Royal Society of Chemistry Information Services, Cambridge, UK, 1991 (cited in EXTOXNET, 1996).

Lewis K, Green A, Tzilivakis J (2007) Pesticide database holding fate and ecotoxicological values. Report DL24 of the FP6 EU-funded FOOTPRINT project. Available at http://www.eu-footprint.org

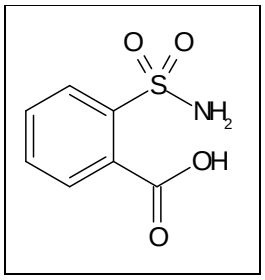
Pesticide Safety Directorate (PSD, 1988) Evaluation on Fluazifop-P-butyl. Available at: http://www.pesticides.gov.uk/PSD_PDFs/Evaluations/010_fluazifop-p-butyl.pdf

Appendix 12.39 2-(Aminosulfonyl) benzoic acid

1 Introduction

2-(Aminosulfonyl) benzoic acid (IN-D5119) is a major degradation product of metsulfuron-methyl (CAS No. 74223-64-6) in soil. The structure of In-D5119 is presented in Figure 1.

Figure 1: Structure of IN-D5119



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of metsulfuron-methyl

Metsulfuron-methyl is the BSI name for methyl 2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)benzoate (IUPIC). It is a herbicide used to control grasses and broad-leaved weeds mainly in cereals and land temporarily removed from production. As a systemic compound with foliar and soil activity, metsulfuron-methyl inhibits plant amino acid synthesis and therefore cell division in the shoots and roots of the plant. It is biologically active at low application rates and works rapidly after take up by the plant. Metsulfuron-methyl is also a predominant metabolite of iodosulfuron-methyl-sodium in soil and drinking water.

Metsulfuron-methyl is sold worldwide often under the product name Ally. It is often supplied as wettable granules and mixed with water to form a spray. The maximum application rate for metsulfuron-methyl is 6 g a.i/ha (PSD, 1995).

2.2 Environmental fate

2.2.1 Parent

Metsulfuron-methyl is highly water soluble (2790 mg/L at 20oC). In soil, the compound is broken down by chemical hydrolysis and microbial degradation. Half-life is typically 10 days under field conditions; breakdown is more rapid at lower soil pH or higher temperatures and at high levels of soil moisture. The major metabolites (fraction >10% of applied rate) are methyl 2-(aminosulfonyl)benzoate (Ref: IN-D5803), 2-(aminosulfonyl) benzoic acid (Ref: IN-D5119), phenylurea (Ref: IN-B5685), and saccharin (Ref: IN-00581).

The sorption Koc for metsulfuron-methyl was measured in the range of 4 – 60 ml/g, indicating very high mobility (EC, 2000).

2.2.2 Metabolite: IN-D5119

No information on the physicochemcial properties or environmental fate of IN-D5119 was identified.

2.3 Potential routes of human exposure

Exposure of humans to metsulfuron-methyl may occur through ingestion of contaminated food and water or through occupational handling or bystander exposure (inhalation or dermal contact).

Although no data on routes of exposure of humans to the metabolite IN-D5119 was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of metsulfuron-methyl, and, to a limited extent, when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of metsulfuron-methyl in humans.

Total recovery of radiolabeled metsulfuron-methyl (dose unknown) administered to rats through the oral route was 91.6 - 103.8 %. The primary route of excretion was via the urine (approx. 71 - 95%) with faecal elimination being 4.8 - 13.3%. Overall, excretion was seen to almost complete within 48 hours. Elimination half-lives (males and females) were 13 - 16 hours for a single low dose, 9 - 12 hours when given as repeat dose over 21-days, and 23 - 29 hours for a single high dose. Tissue burdens were generally minimal (< 0.1% to 1%). Metabolic studies showed that the parent was recovered in both urine and faeces in all treatment groups and was the major contributor to the observed radioactivity (77 - 90% and 1.8 - 6.2% of administered dose, respectively). Four metabolites (saccharin and Metabolites I, II, and III) were found in both matrices and accounted for approximately 5.4 - 8.2% of dose. Two of the metabolites appear to result from sequential hydrolysis reactions terminating in the formation of saccharin, while one was formed by cleavage of the two ring structures (USEPA, 2002).

4 Toxicity Profile

4.1 Metsulfuron methyl

4.1.1 Acute Toxicity

No information on acute toxicity in humans has been identified.

In rats, metsulfuron-methyl has low acute toxicity; LD50 are > 5000 mg/kg bw, > 2000 mg/kg bw and > 5 mg/L for the oral, dermal and inhalation routes respectively. Metsulfuron-methyl did not cause irritation to the eyes of rabbits and did not cause skin sensitisation in Guinea pigs (EC, 2000).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of metsulfuron-methyl in humans was identified.

In a 90 day rat dietary study administering metsulfuron methyl, NOAELs of 68 mg/kg bw/day and 64 mg/kg bw/day for male and females were established. LOAELs of 521 mg/kg bw/day and 659 mg/kg bw/day were established for male and female rats, based on a transient decrease in body weight gain (EC, 2000; full study results unavailable).

In a dermal study in rabbits, a NOAL of 12 mg/kg bw/day and a LOAEL of 500 mg/kg bw/day were established, based on the development of diffuse/multifocal dermatitis. A NOAEL of 125 mg/kg bw/day and LOAEL of 500 mg/kg bw/day were identified for systemic toxicity based on an increased incidence of diarrhoea (EC, 2000; full study results unavailable).

4.1.3 Carcinogenicity and mutagenicity

No information was found on the carcinogenic and mutagenic effects of metsulfuron-methyl in humans.

In the 90 day dietary study described above, no evidence of carcinogenicity was found (EC, 2000; full study results unavailable).

All in vitro and in vivo mutagenicity tests produced negative results (EC, 2000; full study results unavailable).

4.1.4 Reproductive and development toxicity
No information on the reproductive and developmental effects of metsulfuron-methyl in humans was identified.

In a two-generation study in rats, parental systemic NOAELs were 34 mg/kg bw/day for males and 43 mg/kg bw/day for females, based on decreased pre-mating body weight gains by F0 rats in the highest dose group. The NOAELs for reproductive and developmental toxicity were 342 mg/kg bw/day for males and 475 mg/kg bw/day (USEPA, 2002; full study results unavailable).

In rabbits, a parental NOAEL of 25 mg/kg bw/day and a LOAEL of 1000 mg/kg bw/day were established based on increased mortality, decreased body weight gains, and clinical signs of anorexia, red or orange urine and /or exudate. Developmental NOAEL was 700 mg/kg bw/day. No developmental LOAEL was established (EC, 2000; USEPA, 2002; full study results unavailable).

4.2 IN-D5119
No information on the repeat dose toxicity of IN-D5119 in humans was identified.
Searches were made for published information on the toxicity of IN-D5119, including use of the online programme ChemIDPlus (http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp). However, no relevant data were identified.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK identified the presence of several structural alerts and the following conclusions were drawn:

- it is considered plausible (i.e. there is a weight of evidence) that IN-D5119 can cause bladder urothelial hyperplasia in humans. This endpoint is predicted because IN-D5119 is an aryl sulphonamide.

- it is considered plausible (i.e. there is a weight of evidence) that IN-D5119 can cause ocular toxicity in humans. This endpoint is predicted because IN-D5119 is an aryl sulphonamide.

- it is considered plausible (i.e. there is a weight of evidence) that IN-D5119 can cause teratogenicity in humans. This endpoint is predicted because IN-D5119 is an aryl sulphonamide.

- it is considered plausible (i.e. there is a weight of evidence) that IN-D5119 can cause phototoxicity in humans. This endpoint is predicted because IN-D5119 is an aryl sulphonamide.

The analysis by TOPKAT suggested that IN-D5119 was:

- non carcinogenic;
- non mutagenic; and
- not a reproduction toxicant

Table 1 Predicted toxicity data for IN-D5119 using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/ H (range)				
Metsulfuron methyl (experimental data)	>5000	>5	NOAEL 64 mg/kg bw/d Main effect: Transient reduction in body weight gain	Negative	Carcinogenicity potential not established	NOAEL Reproduction: 34 mg/kg/d Developmental: 475 mg/kg bw/d
IN-D5119 (TOPKAT)	10,000 (4700-10,000)	9600 (1200-10,000)	MTD (feed/drink) 914.7 mg/kg MTD (oral gavage) 914.4 mg/kg	Negative	Negative	Indeterminate
IN-D5119 (DEREK)	n/a	n/a	*Equivocal* for bladder urothelial hyperplasia in humans; *Plausible* for phototoxicity and ocular toxicity in humans.	No alert	*Plausible* in mammals	*Plausible* for teratogenicity in humans

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software

5 Guidelines and Standards

5.1 Metsulfuron-methyl

The EC risk classification is: N - Dangerous for the environment: R50, R53. WHO classified metsulfuron-methyl as 'unlikely to present acute hazard in normal use'. Metsulfuron-methyl is classified by EPA as Category III, and must bear the signal word "Caution" on commercial products (Lewis *et al.,* 2007).

An ADI of 0.25 mg/kg bw/day has been established for metsulfuron-methyl based on a carcinogenicity study in rats in which a NOAEL of 25 mg/kg bw/day for non-carcinogenic endpoints was derived; a safety factor (SF) of 100 was applied. (EC, 2000).

5.2 IN-D5119

Due to the absence of any experimental data, it has not been possible to establish a robust experimentally-based NOAEL for IN-D5119, and no ADI has been published

by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

Irrespective of form of administration, an oral MTD of approx. 914 mg/kg bw/day was predicted for IN-D5119 by TOPKAT. Applying a SF of 100 would give a nominal value of 9.14 mg/kg bw/day which is markedly higher than the established ADI of 0.25 mg/kg bw/day for the parent metsulfuron-methyl. Given this, it is – on a highly precautionary basis – therefore proposed to adopt a PSDV of 0.25 mg/kg bw for IN-D5119, i.e. the same value as for the parent; this will provide an overall SF of nearly 3700.

6 References

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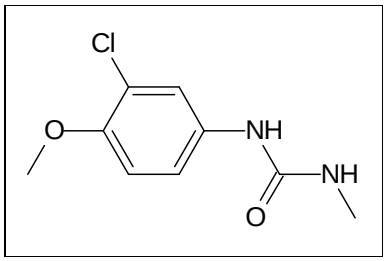
Appendix 12.40 Demethyl Metoxuron

1 Introduction

Demethyl metoxuron is a metabolite of the herbicide metoxuron (*N*'-(3-chloro-4-methoxyphenyl)-*N*,*N*-dimethylurea; CAS No. 19937-59-8).

The structure of demethyl metoxuron is presented in Figure 1.

Figure 1: Structure of demethyl metoxuron



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Metoxuron

Metoxuron is a pre- and post-emergence herbicide used to control some grasses and broad-leaved weeds in carrot and parsnips. Introduced in the 1970s, the active constituent approval, product registrations and MRLs were all withdrawn in 1996 by the APVMA due to concern over the lack of toxicological data. However, the herbicide is currently registered for use in the UK (essential uses only), Netherlands, Hungary and India (PSD, 2009).

2.2 Environmental fate

2.2.1 Parent

Metoxuron has high solubility in water (678 mg/L) and, in assessing the sorption to soil, a Koc value of 120L kg-1 has been described suggesting moderate mobility in the environment (Lewis et al., 2007).

Metoxuron is susceptible to degradation in soil under aerobic conditions, with a degradation half-life of 18.5 days under field conditions. Metoxuron is not considered to be persistent (Lewis et al., 2007).

2.2.2 Metabolite: Demethyl Metoxuron

Demethyl metoxuron is one of the metabolites formed through metabolism or environmental degradation of metoxuron.

No information relating to either the physicochemcial properties or environmental fate of the metabolite demethyl metoxuron was found.

2.3 Potential routes of human exposure

Occupational exposure to metoxuron may occur through inhalation of dust particles and dermal contact during manufacture or use of the herbicide.

Although no data on routes of exposure of humans to the metabolite demethyl metoxuron was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of metoxuron, and, to a limited extent, when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics of parent and metabolite
No information was identified on the toxicokinetics of metoxuron in humans or animals.

No information relating to the toxicokinetics of demethyl metoxuron in humans or animals was identified.

4 Toxicity Profile
4.1 Metoxuron
4.1.1 Acute Toxicity
No information on the acute effects of metoxuron in humans was found.

Experimentally, metoxuron has low acute toxicity in rats; LD50's are 3200 mg/kg, 2000 mg/kg and 5.0 mg/m3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007).

4.1.2 Repeat dose toxicity
No information relating to the repeat dose toxicity of metoxuron in humans or animals was identified.

4.1.3 Carcinogenicity and genotoxicity
No information relating to carcinogenic or genotoxic effects of metoxuron to humans or animals was found.

4.1.4 Reproductive and development toxicity
No information was found on the reproductive and developmental effects of Metoxuron in humans or animals.

4.2 Demethyl Metoxuron
4.2.1 Acute Toxicity
No information on the acute effects of demethyl metoxuron in humans was found.

4.2.2 Repeat dose toxicity
No information was found on the effects of repeat exposure to demethyl metoxuron in humans.

Searches were made for published information on the toxicity of demethyl metoxuron, including use of the online programme ChemIDPlus (US NLM, 2003). However, no additional relevant information was identified.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Analysis by DEREK identified the presence of one structural alert in the molecule leading to the following conclusion being drawn:

- it is considered plausible (i.e. there is a weight of evidence) that demethyl metoxuron will be cause skin sensitisation in humans. This endpoint is predicted because demethyl metoxuron is a phenol or precursor.

The analysis by TOPKAT suggested that demethyl metoxuron was:

- carcinogenic; but

- non-mutagenic

Prediction of the reproductive and developmental toxicity of demethyl metoxuron using TOPKAT was not possible.

Table 1: Predicted toxicity data for Demethyl Metoxuron using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$ (range)				
Metoxuron (experimental data)	3200	5.0	n/d	n/d	n/d	n/d
Demethyl metoxuron (TOPKAT)	45.4 (8.3- 247.6)	212.3 (22.6-2000)	MTD (feed/drink) 74.2 mg/kg MTD (oral gavage) 74.2 mg/kg	Negative	Positive	UE
Demethyl metoxuron (DEREK)	n/a	n/a	*Plausible* that will skin sensitisation in humans	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software; UE – unreliable estimate

5 Guidelines and Standards

5.1 Metoxuron

The EC risk classification is: N - Dangerous for the environment: R50, R53. The EC Safety classification is S60, S61. Metoxuron is classified by WHO as 'unlikely to present acute hazard in normal use'; the herbicide has no EPA classification (Lewis et al., 2007).

Due to the lack of experimental data, no ADI has been proposed by any authoritative organisation for metoxuron (Lewis et al., 2007).

5.2 Demethyl Metoxuron

No ADI has been proposed by any authoritative organisation for Demethyl Metoxuron. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The oral MTD predicted by TOPKAT for Demethyl Metoxuron is 74.2 mg/kg bw/day (regardless of manner of administration). Given the lack of robust repeat dose experimental data for either parent or metabolite and that TOPKAT identified concerns regarding the possible carcinogenicity of the metabolite, it is considered appropriate to include an allowance in the safety factor to reflect the degree of uncertainty. Therefore it is proposed to apply a SF of 1000 to give a PSDV of 0.074 mg/kg bw/day for demethyl metoxuron.

6 References

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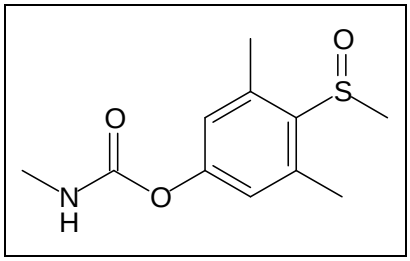
Appendix 12.41 Methiocarb Sulfoxide

1 Introduction

Methiocarb sulfoxide (3,5-dimethyl-4-(methylsulfinyl)phenyl methylcarbamate; CAS No. 2635-10-1) is a metabolite of the insecticide and molluscicide methiocarb (3,5-dimethyl-4-(methylthio)phenyl methylcarbamate; CAS No. 2032-65-7). It is formed within the surrounding soil following application of the parent to plants. It is also formed during the metabolism of absorbed methiocarb in humans.

The structure of methiocarb sulfoxide, is presented in Figure 1.

Figure 1: Methiocarb Sulfoxide



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Methiocarb

Methiocarb has been in use since the 1960s as a molluscicide, acaricide and insecticide for control of snails and slugs in home gardens and ornamentals. It also used on mites, thrips, aphids, leafhoppers, fruit flies, biting insects and some soil pests in field crops. It is also used as a bird repellent on fruit crops (HSDB, 2003).

Methiocarb is sold worldwide under the commercial names Decoy, Draza, Exit Wetex, Huron, Karan and Rivet. It is generally supplied as pellets or granules and applied directly to soil surface (Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Methiocarb has low solubility in water (27 mg/L) and, in assessing the sorption to soil, a Koc value of 660 L kg-1 has been described suggesting slight mobility in the environment (Lewis et al., 2007).

Methiocarb is susceptible to degradation in soil under aerobic conditions, with degradation half-lives of 1.4 and 35 days under laboratory (20oC) and field conditions, respectively. Methiocarb is considered to be moderately persistent (Lewis et al., 2007).

2.2.2 Metabolite: Methiocarb Sulfoxide

Methiocarb sulfoxide is a major metabolites formed through degradation of methiocarb.

In assessing the sorption to soil, a Koc value of 31 mg/L has been described, suggesting mobility in the environment (Lewis et al., 2007).

Methiocarb sulfoxide is susceptible to degradation in soil under aerobic conditions, with a degradation half-life of 3.7 days under laboratory (20oC) conditions. methiocarb is not considered to be persistent (Lewis et al., 2007).

No further information relating to either the physicochemical properties or environmental fate of the metabolite was found.

2.3 Potential routes of human exposure
Occupational exposure to methiocarb may occur through inhalation and dermal contact at workplaces where it produced or used. Monitoring data indicate that the general population may be exposed to methiocarb via ingestion of contaminated food (HSDB, 2003).

Although no data on routes of exposure of humans to the metabolite methiocarb sulfoxide was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of methiocarb, and, to a limited extent, when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics of parent and metabolite
3.1 Methiocarb
No information is available on the toxicokinetics of methiocarb in humans.

In rats administered [ring-1-14C]-methiocarb by oral gavage at 0.25 or 20 mg/kg bw, > 90% of the highest dose was excreted via urine within 48 hr; similar results were obtained at lower doses (Stanley & Johnson 1976). Metabolism involved production of methiocarb phenol, methiocarb sulfoxide phenol and N-hydroxymethyl methiocarb sulfoxide, with methiocarb sulfoxide representing the main metabolic product (Wheeler & Strother, 1971).

No information on to the toxicokinetics of methiocarb sulfoxide was identified.

4 Toxicity Profile
4.1 Methiocarb
4.1.1 Acute Toxicity
No information is available on the acute toxicity of methiocarb in humans.
Methiocarb has high acute toxicity in rats; LD50's are 19 mg/kg, 5000 mg/kg and 0.433 mg/m3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007). Methioicarb is non-irritating to the skin and eyes of rabbits (Crawford & Anderson, 1970). No evidence of skin sensitisation has been seen in Guinea pigs (David, 1988).

4.1.2 Repeat dose toxicity
No information on the effects following repeated methiocarb exposure of humans was found.

Experimentally, chronic exposure effects were assessed in Wistar TNO W.74 rats administered a diet containing methiocarb at up to 600 ppm (equivalent to 29 and 42 mg/kg bw/day, for males and females respectively) for 2 years. At 600 ppm, weight gain was reduced throughout period of treatment. Several changes in haematological parameters were reported, comprising increased leukocyte and reticulocyte counts in females at 600 ppm and 200 ppm after three months. Mean corpuscular haemoglobin concentration was also reduced in males at 600 ppm and elevated leukocyte counts was apparent in males at 67 and 200 ppm. After 12 months, mean corpuscular haemoglobin concentration was decreased in males at the intermediate doses, but was increased in females at 600 ppm. An increased reticulocyte count was seen in females at 600 ppm. By 24 months, no compound-related changes in haematology were seen but biochemical parameters were disturbed. Total protein concentration was raised in females at 200 and 600 ppm and in all treated male

groups following 6 and 12 months of treatment respectively; at study termination, no increase in total protein concentration was noted in either sex,. However, changes at 600 ppm at this time included increased alanine aminotransferase (ALAT) activity in treated females and plasma urea and depressed cholinesterase activity in both sexes. The systemic NOEL was 600 ppm (30 mg/kg bw/day) based on body weight decreases; these which were considered to be a secondary effect of cholinesterase inhibition. The NOEL for cholinesterase inhibition was 67 ppm (3.35 mg/kg bw/day; Krötlinger et al., 1981; Krötlinger, 1990).

In a further study, Beagle dogs were fed methiocarb in the diet at up to 240 ppm (equivalent to 6 mg/kg bw/day) for two years. The only clinical findings were mild weakness of the hind limbs, trembling, reduced alertness and some vomiting at the highest dose during the first 14 weeks of the study. Reflexes and ophthalmic parameters were, however, normal. At the highest dose, food intake was reduced in both sexes and additionally in females at the intermediate dose; however, there was no affect on body weight. Haematological and most biochemical parameters were unaffected by treatment. However, plasma cholinesterase activity was depressed at ≥ 15 ppm although erythrocyte and brain acetylcholinesterase activity was not consistently inhibited. The NOAEL was 60 ppm (equivalent to 1.5 mg/kg bw/day) based on clinical signs (Hoffman & Schilde, 1980).

4.1.3 Carcinogenicity and genotoxicity

No information on the carcinogenic or genotoxic potential of methiocarb in humans was identified.

Carcinogenicity was assessed in the 2 year feeding study in Wistar rats described above. No evidence of carcinogenicity was found Krötlinger et al., 1981; Krötlinger, 1990).

Methiocarb was negative in a number of in vitro and in vivo mutagenicity assays (JMPR, 1998).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of methiocarb in humans was identified.

In a three-generation study, rats administered methiocarb via the diet at up to 300 ppm showed no adverse effects on fertility, litter size, pup birth weight, offspring survival or lactation (JMPR, 1998).

In FB 30 rats orally dosed at up to 10 mg/kg bw/day on days 6 – 15 of gestation, maternal toxicity was apparent at the highest dose (reduced weight gain) but no teratogenic or fetotoxic effects were observed. A developmental toxicity NOAEL of 10 mg/kg bw/day was established (Lorke, 1971).

4.2 Methiocarb Sulfoxide

4.2.1 Acute Toxicity

No information on the acute toxic effects of methiocarb sulfoxide in humans or animals was identified.

4.2.2 Repeat dose toxicity

No information was found on the repeat dose toxicity of methiocarb sulfoxide in humans was identified.

Searches were made for published information on the toxicity of methiocarb sulfoxide, including use of the online programme ChemIDPlus (US NLM, 2003). However, no relevant information was identified. Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1: Predicted toxicity data for methiocarb sulfoxide using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$ (range)				
Methiocarb (experimental data)	19	5000	NOAEL: 1.5 mg/kg/day (dogs)	Negative	Negative	Negative
Methiocarb Sulfoxide (TOPKAT)	17.9 (2.7-117.1)	897.1 (92-8700)	MTD (feed/drink) 19.3 mg/kg MTD (oral gavage) 19.3 mg/kg	Negative	Negative	Indeterminate
Methiocarb Sulfoxide (DEREK)	n/a	n/a	*Plausible* for cholinest-erase inhibition in humans	*Plausible* for chromosome damage in humans	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software

DEREK concluded the presence of two structural alerts and the following conclusions were drawn:

it is considered plausible (i.e. there is a weight of evidence) that methiocarb sulfoxide will be cause cholinesterase activity in humans. This endpoint is predicted because methiocarb sulfoxide is an N-Methyl or N,N-dimethyl carbamate.

it is considered plausible (i.e. there is a weight of evidence) that methiocarb sulfoxide will cause chromosome damage in humans. This endpoint is predicted because methiocarb sulfoxide is an Aryl N-alkylcarbamate.

The analysis by TOPKAT suggested that methiocarb sulfoxide was:

non-carcinogenic; and
non-mutagenic.

Prediction of the reproductive and developmental toxicity of methiocarb sulfoxide using TOPKAT was not possible.

5 Guidelines and Standards
5.1 Methiocarb
The EC risk classification is: T – Toxic: R25: N - Dangerous for the environment: R50, R53. The EC Safety classification is S1/2, S22, S37, S45, S60, S61. Methiocarb is classified by WHO as 'highly hazardous' and by the US EPA as 'highly toxic' (Lewis *et al*., 2007).

An ADI of 0.013 mg/kg bw/day has been established for methiocarb using a safety factor (SF) of 100, based on NOAEL of 1.5 mg/kg bw/day from a 2-year dog feeding study (Lewis *et al*., 2007).

5.2 Methiocarb sulfoxide
No ADI has been proposed by any authoritative organisation for methiocarb sulfoxide. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

Overall, the toxicity profile for methiocarb sulfoxide is predicted to be similar to that of the parent. Although DEREK drew attention to the possibility of possible chromosomal effects, no such alert was triggered for TOPKAT and the parent has been found to be non-mutagenic in *in vivo,* as well as *in vitro,* assays suggesting such a genotoxic potential may be unlikely. In any event, the predicted oral MTD by TOPKAT for methiocarb sulfoxide is 19.3 mg/kg bw/day (irrespective of form in which dose is supplied by the oral route). Applying a SF of 100 would give a nominal value of 0.19 mg/kg bw/day which is approximately 10-fold above the established ADI of 0.013 mg/kg bw/day for the parent methiocarb. Given these considerations, it is therefore proposed to adopt a PSDV of 0.013 mg/kg bw/day for methiocarb sulfoxide, i.e. the same value as for the parent. This would provide an overall SF of approximately approx 1500.

6 References
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Wheeler L & Strother A. (1971) In vitro metabolism of the N-methylcarbamates, Zectran and Mesurol, by liver, kidney and blood of dogs and rats. Journal of Pharmacology and Experimental Therapy, 178, 371-382 (cited in JMPR, 1998).

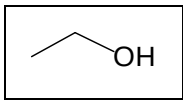
Appendix 12.42 Ethanol

1 Introduction

Ethanol (ethyl alcohol; CAS No. 64-17-5) can be formed as a metabolite of the fungicide fosetyl-aluminium (aluminium tris-*O*-ethylphosphonate; CAS No. 39148-24-8) within the plant and surrounding soil. It is also formed during the metabolism of absorbed fosetyl-aluminium in humans. Ethanol is also used in its own right post-harvest as a catalytic generator for the production of ethylene gas for fruit ripening and as a bactericide (topical disinfectant) and in a wide range of other industrial and consumer applications.

The structure of ethanol is presented in Figure 1.

Figure 1: Structure of ethanol



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Fosetyl-aluminium

Fosetyl-aluminium is a systematic fungicide used for both preventative and curative activities against Oomycetes, Alternaria and Pennicillium on avocado, cacao, citrus, hops, ornamentals, pineapple, rubber, strawberries, fruit crops, tobacco, vegetable crops and vines. It is also used to suppress bacterial pathogens such as fireblight (eawinia) on pome fruit, xanthomonas and on ornamentals (HSDB, 2006).

Fosetyl-aluminium is sold worldwide under the commercial names Aliette 80 WG and Fullstop; it is generally sold as wettable granules that are mixed with water and applied as a spray or used as a seed treatment (Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Fosetyl-aluminium has high solubility in water (110,000 mg/L) and, in assessing the sorption to soil, a Koc value of 1703 L kg-1 has been described suggesting slight mobility in the environment (Lewis et al., 2007).

Fosetyl-aluminium is highly susceptible to degradation in soil under aerobic conditions, with degradation half-lives of 0.1 and 0.04 days under laboratory (20oC) and field conditions respectively; Fosetyl-aluminium is not considered to be persistent (Lewis et al., 2007).

2.2.2 Metabolite: Ethanol

Ethanol is a major metabolite formed through degradation of fosetyl-aluminium with an estimated maximum occurrence fraction of 0.780 (78%; Lewis et al., 2007).

Ethanol is miscible in water and, in assessing the sorption to soil a Koc value of 1 L kg-1 has been described suggesting high mobility in the environment (Lewis et al., 2007).

2.3 Potential routes of human exposure

Occupational exposure to Fosetyl-aluminium may occur through inhalation and dermal contact with this compound at workplaces where Fosetyl-aluminium is produced or used (HSDB, 2006a). The general population may be exposed to the

fungicide via dermal contact, inhalation of ambient air or through ingestion of drinking water and food containing Fosetyl-aluminium (HSDB, 2006a).

Exposure of humans to ethanol as a metabolite of fosetyl-aluminium may similarly occur through ingestion of this metabolite in food and water contaminated with fosetyl-aluminium and its metabolites (oral route) or during the direct metabolism of any absorbed parent fungicide.

3 Toxicokinetics of parent and metabolite

3.1 Fosetyl-aluminium

Toxicokinetic studies have been carried out in Sprague-Dawley rats administered a single oral dose of 3000 mg/kg radiolabeled fosetyl-aluminium. Absorption was seen to be extensive and excretion rapid, mainly occurring within 24 hours following administration. Absorbed fosetyl-aluminium was distributed widely, with highest levels of activity in kidneys, liver, lungs, spleen, fat, adrenals, gonads and tissues with high metabolic activity. The main routes of excretion were through exhaled air (50%) and urine (32 – 33%), with faecal excretion accounting for 1.85% and 3.3% in males and females respectively, 168 hours following administration (EFSA, 2005).

Metabolism studies in rat have shown that fosetyl-aluminium is metabolised to phosphorous acid and ethanol which is subsequently converted via acetaldehyde and acetate to carbon dioxide. The phosphorous acid produced is excreted largely unchanged although limited oxidation to phosphate may also occur (ECB, 2000).

3.2 Ethanol

The toxicokinetics of ethanol has been extensively studied in both humans and animals, and as a consequence the processes are well defined. Due to the wealth of information available, a summary is given below without specific experimental details. Following oral administration, ethanol is rapidly absorbed into the bloodstream from the stomach and small intestines; approximately 80% of administered dose is absorbed in the small intestine, with the remainder being absorbed in the stomach. Overall, 80 - 90% of absorption occurs within 30 - 60 min in a healthy adult (Ellenhorn, 1988). Ethanol absorption can also occur following exposure by inhalation; approximately 62% of dose may be absorbed via this route (Bingham, 2001).In contrast, absorption via the skin is low, with only around 1% absorbed (Bingham, 2001).

Irrespective of the route of exposure, following absorption into the bloodstream, ethanol is distributed throughout the body (Meulenberg & Vijverberg, 2000) with the final volume of distribution close to that of total body water (Endres & Bruner, 1994). Ethanol perfuses organs with the greatest blood supply most quickly (brain, lungs and liver) and equilibrium between tissues and blood is generally achieved within 1 – 1.5 hr after ingestion (Gossel & Bricker, 1994; Martin et al., 1984).

Prior to absorption, ingested ethanol undergoes limited metabolism (first pass metabolism) in the stomach by gastric alcohol dehydrogenase (ADH; Lim et al., 1993; Roine et al., 1991). The role of first-pass metabolism, however, is of less relevance for exposure to ethanol via inhalation and dermal routes. Once absorbed, ethanol is metabolised, principally by the liver (92 - 95 %; Norberg et al., 2003) with a small proportion of metabolism occurring in other tissues such as the kidney and lung (Crabb et al., 1987; Lieber & DeCarli, 1977). In the liver, ethanol metabolism is in three steps: (i) oxidation of ethanol to acetaldehyde (AcH) (ii) conversion of AcH to acetate, and (iii) oxidation of acetate to carbon dioxide and water. In the first step, ethanol is converted to AcH by alcohol dehydrogenase (ADH); this occurs in the

soluble fraction of liver cells (cytosol). In addition, approximately 6% of ethanol is oxidised by the microsomal ethanol oxidising system (MEOS) which is located in the smooth endoplasmic reticulum and a very small proportion by hepatic catalase in peroxisomes. The conversion of ethanol to AcH by ADH is the rate-limiting step in ethanol metabolism. Pulmonary tissue also contains microsomal oxygenase enzymes (flavin and cytochrome P450) but at lower levels than found in liver (Bond, 1990). Similarly, skin has many of the enzymes that occur in the liver but its metabolising potential is considered too small to be considered of significance for most chemicals at an estimated 2% of that of the liver (Pannatier et al., 1978).

The majority of absorbed ethanol is eliminated from the body by metabolism (95 –-98 %; Norberg et al., 2003) . A small amount of ethanol (2 – 5 %) is also eliminated unmetabolised in breath, urine and sweat (Holford, 1987; Norberg et al., 2003).

4 Toxicity Profile

4.1 Fosetyl-aluminium

4.1.1 Acute Toxicity

Fosetyl-aluminium is a severe eye irritant in humans (US EPA, 1990). No information on the acute toxicity of fosetyl-aluminium to humans was identified.

Experimentally, fosetyl-aluminium has low acute toxicity in rats; LD50's are 7080 mg/kg, 2000 mg/kg and 5.11 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007).

In animals, fosetyl-aluminium is non-irritating to the skin and no evidence of skin sensitisation has been reported (EFSA, 2005).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of fosetyl-aluminium in humans was identified.

Experimentally, in a two-year feeding study in Sprague-Dawley rats fed a diet containing fosetyl-aluminium at levels up to 30,000 ppm (equivalent, at the highest dose, to 1372 and 1786 mg/kg bw/day, for males and females respectively), no treatment-related effects on body weight, ophthalmology, haematology or clinical chemistry were reported (CEPA, 1998). A NOAEL of 348 and 450 mg/kg bw/day was proposed for males and females respectively.

Chronic exposure to fosetyl-aluminium was also assessed in Beagle dogs fed a diet containing up to 40,000 ppm (equivalent, for the high dose group, to 1228 and 1190 mg/kg bw/day for males and females respectively) for two years. No consistent treatment-related effects on body weight, food consumption, ophthalmology, haematology, clinical chemistry, or urinalysis were reported. Histopathogy showed increased incidence of seminiferous tubule degeneration in treated males consisting of spermatocytic and/or spermatidic giant cells in the lumen of the seminiferous tubules; lesions were more numerous at 40,000 ppm than at 20,000 ppm, although the degree of severity was similar. In females, an increase in the incidence and severity of vacuolar tubular lesions of the kidney was reported with increasing dose. A NOEL of 10,000 ppm (348 and 450 mg/kg bw/day in males and females respectively) was proposed based on testicular effects in males (CEPA, 1998).

4.1.3 Carcinogenicity and genotoxicity

No information on the carcinogenic or mutagenic toxicity of fosetyl-aluminium in humans was identified.

In the chronic feeding study in rats described above, a dose-related increase in incidence of pheochromocytoma of the adrenal medulla was reported in males, with an associated decrease in incidence of focal hyperplasia; the effect on tumour incidence was considered treatment-related. In high dose males, urinary bladder neoplasia (carcinoma and papilloma combined) and transitional cell hyperplasia were also increased (CEPA, 1998).

The genotoxicity/ mutagenicity of fosetyl-aluminium has been assessed in a number of in vivo and in vitro assays; all assays have been negative (HSDB, 2006).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of fosetyl-aluminium in humans was identified.

In a three-generation reproductive toxicity study, CFY rats were fed diets containing fosetyl-aluminium at up to 24,000 ppm. At the highest dose, seven deaths were recorded in the F1B and three in the F2B generations; autopsy showed that these animals had haemorrhage of the urinary bladder wall, increased renal pelvic dilatation, interstitial nephritis and papillary necrosis. Body weight gain was reduced in both sexes at the highest dose, this was especially apparent in F1B and F2B generation males. Histopathology showed minimal epithelial hyperplasia and/or hypertrophy of the transitional epithelium of the urinary bladder, sometimes associated with small papillary projections and/or desquamation of epithelial cells in the lumen, for both sexes of F3B animals. No effects on reproductive parameters were seen and a reproductive NOEL of 24,000 ppm was proposed (CEPA, 1998).

Developmental toxicity of fosetyl-aluminium was assessed in pregnant CFY rats by oral administration of up to 4000 mg/kg on days 6 to 15 of gestation. Maternal toxicity was apparent as reduced body weight gain and fetotoxicity as reduced viability of offspring. No adverse developmental effects were reported. A developmental NOEL of 1000 mg/kg was proposed (CEPA, 1998).

Developmental toxicity of fosetyl-aluminium was further assessed in pregnant New Zealand White rabbits subject to oral gavage at up to 500 mg/kg from day 6 to 16 of gestation. No developmental effects were reported (CEPA, 1998).

4.2 Ethanol

4.2.1 Acute Toxicity

In humans, acute exposure to low levels of ethanol through ingestion results initially in CNS stimulation with associated behavioural changes including relaxation and talkativeness. At higher levels, ethanol exerts a depressant effect on the CNS, altering motor activity and inducing sleep, with convulsions and coma occurring if very high levels are reached (Eckardt, et al., 1998; Fadda & Rossetti, 1998; Pohorecky & Brick, 1988).

Experimentally, ethanol has low acute toxicity in rats; LD50's are 6000 mg/kg, 20,000 mg/kg and 3.9 mg/m3 by the oral, dermal or inhalation routes respectively (EFSA, 2005).

Ethanol vapour is a physiological irritant to the eyes and to the upper respiratory tract (ACGIH, 2001) Dermal contact with high concentrations of ethanol, such as those encountered in an occupational setting, may result in stinging and burning sensations if skin is broken (ACGIH, 2001).

4.2.2 Repeat dose toxicity

Chronic exposure to moderate or high levels of ethanol through oral consumption has been principally linked to development of diseases of the liver in both humans and animals; this has been extensively reviewed elsewhere (ACGIH, 2000; 1987; Baan et al., 2007; IARC, 1988). A NOAEL of approximately 2400 mg/kg bw/day has been proposed from a long-term dietary study in rats, based on minor changes to organ weights and haematology in males and biochemistry in males and females; increase in oestrous cycle length was seen in females (INCHEM, 2004)

4.2.3 Carcinogenicity and genotoxicity

Evidence of the carcinogenicity of ethanol, largely based on studies investigating the effects of ethanol in alcoholic beverages, in humans and animals has recently been considered by the IARC Working Group which has led IARC to classify ethanol in alcoholic beverages as, carcinogenic to humans, i.e. a Group 1 carcinogen (Baan et al., 2007). However, ethanol per se has not been classified as a human carcinogen.

On balance ethanol is not considered to be genotoxic. Negative results from a number of bacterial mutation assays appear to be reliable. Of the mammalian cell mutation assays a weak mutagenic effect in mouse lymphoma cells occurred only at very high ethanol concentrations. In vivo tests for chromosome aberrations in both rats and Chinese hamsters have given negative results. There is very little evidence to suggest that ethanol is genotoxic in somatic cells and it may have a very limited capacity to induce genetic changes in vivo but under very specific circumstances and at very high doses achievable in humans only by deliberate oral ingestion (INCHEM, 2004).

4.3.4 Reproductive and development toxicity

Ethanol, in association with alcoholic beverage, is considered to be a teratogen and to exert other reproductive effects such as foetal alcohol syndrome (FAS). FAS is a well documented health effect caused by high maternal alcohol ingestion (greater than 90g per day) during pregnancy, resulting in permanent physiological changes to the foetus (Jones et al., 1973).

Additional information was also sought through application of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Summaries of the toxicity data predicted for Ethanol from these tools are summarised below (Table 1).

DEREK identified the presence of one structural alert and the following conclusion was drawn:

- it is considered certain (i.e. there is evidence) that ethanol will be cause teratogenic effects in humans. This endpoint is predicted because ethanol is an alkyl alcohol.

The analysis by TOPKAT suggested that ethanol was:

- non-carcinogenic;
- non-mutagenic; but
- a developmental toxicant

Table 1: Predicted toxicity data for ethanol using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Fosetyl-aluminium (experimental data)	7080	5.11	NOAEL: 300 mg/kg/day (rat and dog)	Negative	Negative (humans)	Negative
Ethanol (experimental data)	6000	39	NOAEL: 2400 mg/kg/day	Negative	Negative	Developmental toxicant (humans)
Ethanol (TOPKAT)	2800 (967.8-8200)	10,000 (10,000-10,000)	MTD (feed/drink) 452.2 mg/kg MTD (oral gavage) 1200 mg/kg	Negative	Negative	Developmental toxicant
Ethanol (DEREK)	n/a	n/a	No alert	No alert	No alert	Certain teratogen in humans

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/s – not suitable for use with software; n/d – not determined; n/a – prediction not applicable to software;

5 Guidelines and Standards

5.1 Fosetyl-aluminium

The EC risk classification is: Xi - Irritant: R41. The EC Safety classification is S2, S26, S39, S46. Fosetyl-aluminium is classified by WHO as 'unlikely to present acute hazard in normal use' and by the US EPA as 'slightly toxic' (Lewis *et al*., 2007).

An ADI of 3 mg/kg bw/day has been established for fosetyl-aluminium using a safety factor (SF) of 100, based on two-year feeding studies in the rat and dog (EFSA, 2005).

5.2 Ethanol

The EC risk classification is: H – Handling risk: R11. The EC Safety classification is S2, S7, S16. Ethanol is not classified by WHO or by the US EPA (Lewis *et al*., 2007).

No ADI has been proposed by any authoritative organisation for ethanol. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

A NOAEL of 2400 mg/kg bw/day has been proposed from a repeat dose toxicity dietary study in rats (INCHEM, 2004). Applying a safety factor (SF) of 100 would give an ADI of 24 mg/kg bw/day. It is therefore, – on a highly precautionary basis proposed to adopt a PSDV of 24 mg/kg bw/day for ethanol.

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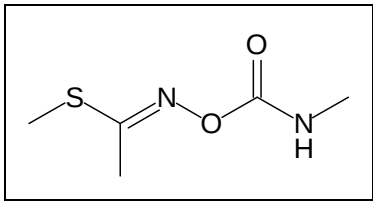
Appendix 11.43 Methomyl

1 Introduction

Methomyl (methyl *N*-[[(methylamino)carbonyl]oxy]ethanimidothioate; Cas No. 16752-77-5) is a metabolite of thiodicarb (dimethyl *N*,*N*'-[thiobis[(methylimino)carbonyloxy]]bis[ethanimidothioate]; Cas No. 59669-26-0) an insecticide, molluscicide and ovicide. Methomyl is also an insecticide and acaricide in its own right.

The structure of methomyl is presented in Figure 1.

Figure 1: Structure of methomyl



2 Use and environmental fate of parent and metabolite and potential human exposure routes

2.1 Use

2.1.1 Thiodicarb

Thiodicarb has been in use since the 1980s as an insecticide, molluscicide and ovicide for control of slugs in cereals and oilseed rape (PSD, 1992).

Thiodicarb is sold mainly in the UK and Australia under the commercial names Judge and Toro; it is generally supplied as ready to use bait (Lewis et al., 2007).

2.1.2 Methomyl

Methomyl has been used since 1968 as an insecticide and acaracide for the control of a wide range of insects and spider mites in fruit, vegetables, olives, hops, vines, ornamentals, field crops, flax, cotton, tobacco and soy beans (Tomlin, 1997).

Methomyl is sold worldwide under the commercial name methomyl 20SC; it is generally supplied as a soluble concentrate that is mixed with water and used as a spray (Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Thiodicarb has low solubility in water (22.2 mg/L) and, in assessing the sorption to soil, a Koc value of 418 L kg-1 has been described suggesting moderate mobility in the environment (Lewis et al., 2007).

Thiodicarb is susceptible to degradation in soil under aerobic conditions with degradation half-lives of 0.39 and 18 days under laboratory (20^{o}C) and field conditions respectively. Thiodicarb is not considered to be persistent (Lewis et al., 2007).

Methomyl is formed as the major breakdown product of thiodicarb with an estimated maximum occurrence fraction of 0.80 (80%; Lewis et al., 2007).

2.2.2 Metabolite: Methomyl

Methomyl has high solubility in water (55000 mg/L). In assessing the sorption to soil, a Koc value of 25.2 L kg-1 has been described suggesting it to be mobile in the environment (Lewis et al., 2007).

Methomyl is susceptible to degradation in soil under aerobic conditions with a degradation half-life of 6.97 under laboratory (20oC) conditions. Methomyl is not considered to be persistent (Lewis et al., 2007).

2.3 Potential routes of human exposure

Occupational exposure to thiodicarb may occur via dermal contact and inhalation during and after normal use of liquid and wettable powder formulations. Monitoring data indicate that the general population may be exposed to thiodicarb via ingestion of food products and water containing thiodicarb (US EPA, 2001).

Occupational exposure to methomyl may also occur through inhalation of dust and dermal contact during occupational use. The general population may be exposed to methomyl as a result of ingestion of food products or water containing methomyl or thiodicarb and its metabolites, or during the direct metabolism of any absorbed parent compound.

3 Toxicokinetics of parent and metabolite

3.1 Thiodicarb

Toxicokinetic studies have been carried out in rats given a single oral dose of [14C]-thiodicarb at 4 or 16 mg/kg. Thiodicarb was rapidly absorbed and distributed within five minutes of administration. After 96 hours, 48% of radioactivity had been eliminated in respiratory gases, 32% in urine and 4.5% in faeces; the remaining activity was associated with the carcass (Hayes and Laws, 1991).

In Sprague-Dawley rats given a single oral dose of [14C]-thiodicarb at 16 mg/kg, thiodicarb metabolism proceeded via formation of methomyl which was subsequently converted to methomyl methylol, oxime, sulfoxide and sulfoxide oxime. These unstable intermediates were subsequently converted to acetonitrile and carbon dioxide which were eliminated primarily by exhalation and in urine; a small fraction of acetonitrile was further degraded to acetamide, acetic acid and carbon dioxide (Tomlin, 1994).

3.2 Methomyl

In rats administered a single oral dose of 5 mg/kg 14[C]-methomyl, near complete absorption (95 – 98%) was seen. Metabolism proceeded via rapid conversion of methomyl to methomyl methylol, oxime, sulfoxide and sulfoxide oxime with subsequent conversion of intermediates to acetonitrile and carbon dioxide; elimination of these products was primarily via exhalation and the urine. Within 24 hours of dosing, > 90% of radioactivity wad eliminated, 25% via urine, 25% as CO2 and 50% by exhalation as acetonitrile (ACGIH, 2001).

4 Toxicity Profile

4.1 Thiodicarb

4.1.1 Acute Toxicity

No information on the acute effects of Thiodicarb on humans was found.

Thiodicarb is non-irritating to the skin and eyes of rabbits. No evidence of skin sensitisation has been noted in Guinea pigs (PSD, 1992).

Experimentally, Thiodicarb has high acute toxicity in rats; LD50's are 50 mg/kg, 2000 mg/kg and 0.66 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007).

4.1.2 Repeat dose toxicity

No information on the effects of repeated exposure to thiodicarb of humans was found.

In F344 rats fed diets containing thiodicarb at up to 10 mg/kg bw/day, reduced body weight gain and increased in incidences of nonneoplastic lesions (including pituitary cysts, thymic epithelial hyperplasia and hemosiderosis of the mediastinal lymph nodes) were apparent at the highest dose. At doses of ≥ 3 mg/kg bw/day, males showed an increased incidence of prostatitis and hepatocellular hyperplasia. A NOEL of 3 mg/kg bw/day was established based on decreased body weight (Hayes, 1991).

In a two year feeding study in mice, no effects were seen on food consumption or body weight gain at up to 10 mg/kg bw/day. During the final two months of the study, mortality was increased at 10 mg/kg bw/day; a NOEL of 3 mg/kg bw/day was established (Hayes, 1991).

In dogs fed diets containing thiodicarb at up to 1500 ppm (equivalent to 38.3 mg/kg bw/day for males and 39.5 mg/kg bw/day for females), for one year, toxic effects included increased body weight-relative liver and spleen weights and decreased plasma acetylcholinesterase activity at the highest doses. A NOEL of 487 ppm (equivalent to 12.8 and 13.8 mg/kg bw/day for males and females respectively) was established (Hayes, 1991).

4.1.3 Carcinogenicity and genotoxicity

No information on the carcinogenic or genotoxic potential of thiodicarb in humans was identified.

In the two year rat chronic feeding study described above, thiodicarb did not result in an increase in incidence of neoplastic lesions (HSDB, 2002).

Thiocarb was negative in both in vitro and in vivo mutagenicity assays and is, therefore, considered not to be a mutagen (PSD, 1992).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of thiodicarb in humans was identified.

In a three-generation study, F344 rats fed thiodicarb containing diet at levels designed to achieve doses of up to 10 mg/kg bw/day, parental effects comprised a slight reduction in body weight gain in treated F0 males but no associated reduction in food consumption. No effect on fertility, gestation or gestation survival indices or on pup weight was seen. Pup viability, 14 day survival rates and lactation indices were also unchanged. A NOEL of 10 mg/kg bw/day was established for reproductive toxicity; however, no NOEL for developmental endpoints was cited (PSD, 1992).

In a further study, male rats administered thiodicarb in the diet at levels resulting in achieved doses of 3.98 or 1.99 mg/kg bw/day for 65 days, decreased sperm cell count, sperm motility and live/dead sperm ratio were noted (Amer, 1996).

The developmental toxicity of thiodicarb was assessed in female albino rats administered oral doses of 3.98 or 1.98 mg/kg bw/day on gestation days 6 – 15. Growth retardation of the foetuses and multiple malformations of related viscera (including dilated cerebral ventricles, absence of thymus, atrophy of lungs and heart and dilated renal pelvis) and skeleton (incomplete ossification of the skull, reduction in number of caudal and sacral vertebrae, absence of some sternebrae, incomplete pelvic girdle, fused tibia and fibula and absence of phalanges) were noted; however no NOAEL was cited for developmental effects (Amer, 1996).

4.2 Methomyl

4.2.1 Acute Toxicity

Acute exposure of humans to methomyl may result in symptoms typical of cholinesterase inhibition; these included nausea, miosis, headache, lacrimation, salivation, vomiting and abdominal pain. Diminished respirations, hypotension, muscle fasciculations, frothing from the mouth and tachycardia have also been observed. Deaths following ingestion of methomyl have been reported (Ellenhorn, 1997).

Experimentally, methomyl has high acute toxicity in rats; LD50's are 30 mg/kg, 2000 mg/kg and 0.215 mg/m^3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007).

In rats, methomyl is an irritant to the pulmonary system, and may produce tremors, irregular breathing, grooming action, salivation, lacrimation and bulging eyes with a red discharge (HSDB, 2003).

4.2.2 Repeat dose toxicity

No information on the repeat dose toxicity of methomyl in humans was identified.

Experimentally, in rats fed diets containing methomyl at doses up to 400 mg/kg bw/day for 22 months, male rats showed decreased growth over the first year which associated with lower food consumption at 200 and 400 mg/kg bw/day. Lower growth rate was also noted in males at 200 mg/kg bw/day and females at 400 mg/kg bw/day over the first year but was not statistically significant. At 18 and 22 months, there was a trend towards lower haemoglobin values in treated females and an increase incidence and severity of extra medullary haematopoiesis was noted in the spleens of females given 200 or 400 mg/kg bw/day. Renal epithelial vacuolization and proximal convoluted tubule hypertrophy were noted in both sexes at 400 mg/kg bw/day. A NOEL of 100 mg/kg bw/day was established based on effects on body weight and hematopoietic changes (WHO, 1996).

In a two year dog study in which animals were fed diet containing methomyl at levels up to 1000 ppm (equivalent for the high level to doses of 31.12 mg/kg bw/day for males and 32.67 mg/kg bw/day for females) signs of acetylcholinesterase inhibition, increased mortality, slight to moderate anemia and compensatory haematopoiesis in the spleen and bone marrow, and haemosiderin deposits, epithelial swelling of kidney tubules and minimal to slight bile-duct proliferation were noted at the highest dose. At 400 ppm (equivalent to 10.93 mg/kg bw/day for males and 13.90 mg/kg bw/day for females) accumulation of pigment in the spleen and kidneys was noted. No effects were seen on body weight, food consumption, blood acetylcholinesterase activity or serological or urological parameters. A NOEL of 100 ppm (2.94 mg/kg bw/day for males and 2.31 mg/kg bw/day for females) was established (Hayes, 1991).

4.2.3 Carcinogenicity and genotoxicity

No information relating to carcinogenic or genotoxic potential of methomyl to humans was identified.

In the 2 year feeding studies in rats and dogs reported above, no effects on the incidence of neoplastic lesions were noted (HSDB, 2003).

The genotoxicity of methomyl has been assessed in a number of genotoxicity and mutagenicity assays. Methomyl induced chromosome aberrations in mammalian cells in both in vitro and in vivo assays (Wei, 1997) and induced DNA damage in Swiss CD1 mice through an indirect mechanism (Bolognesi et al., 1994). However, methomyl was negative in an Ames test with and without metabolic activation (Blevins et al., 1977).

4.2.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of methomyl in humans was identified.

In a three-generation study, rats fed diet containing methomyl at 50 or 100 ppm showed no adverse effects on reproductive parameters (NRC, 1977).

In rats given an oral dose of 17 mg/kg bw/day for 2 months, effects of methomyl on hormonal, histopathological and histochemical changes in the testes was assessed. In treated animals, a significant decrease in the level of testosterone and significant increases in level of FSH, LH and prolactin were observed. Histopathologically, the testes of treated rats showed variable degrees of degenerative changes in the seminiferous tubules (including total cellular destruction). Histochemical analysis found significantly increased activity of acid phosphatase and alpha esterase enzymes and a significant reduction in succinic dehydrogenase enzyme activity. The hormonal changes and testicular damage were considered persistent, being still apparent after 30 days following withdrawal of treatment. It was therefore suggested that chronic exposure to methomyl may have a deleterious effect on the testes although the impact on reproductive performance is unclear (Mahgoub and El-Medany, 2001).

Developmental toxicity of methomyl was assessed in New Zealand White rabbits fed dietary levels of up to 100 ppm on gestation days 8 – 16. No evidence of teratogenic effects was seen (NRC, 1983).

Additional information was sought through use of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are are summarised below (Table 1).

DEREK identified the presence of one structural alert and the following conclusion was drawn:

- it is considered plausible (i.e. there is a weight of evidence) that methomyl will be cause cholinesterase inhibition in humans. This endpoint is predicted because methomyl is an N-Methyl or N,N-dimethyl carbamate.

The analysis by TOPKAT suggested that methomyl was:

- non-carcinogenic; and

- non-mutagenic.

Prediction of the reproductive and developmental toxicity of methomyl was not possible using TOPKAT.

Table 1: Predicted toxicity data for methomyl using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Thiodicarb (experimental data)	50	0.66	NOAEL: 12.8 mg/kg/day (dogs)	Negative	Negative	Increased incidence of delayed ossification (rats)
Methomyl (experimental data)	30 (Rat)	0.215 (Rat)	NOAEL: 2.6 mg/kg bw/day (dogs - feed) NOAEL: 100 mg/kg bw/day (dogs - feed)	n/d	Negative (rat/dog)	Negative (rat) Testicular toxin (dog)
Methomyl (TOPKAT)	22.6 (4-129.7)	1700 (83-10,000)	MTD (feed/drink) 0.829 mg/kg MTD (oral gavage) 2.3 mg/kg	Negative	Negative	UE
Methomyl (DEREK)	n/a	n/a	*Plausible* for cholinest-erase inhibition in humans	No alert	No alert	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software; UE – unreliable estimate

5 Guidelines and Standards

5.1 Thiodicarb

The EC risk classification is: T – Toxic: R23, R25: Xi – Irritant: R43; N - Dangerous for the environment: R50, R53. The EC Safety classification is S2, S61, S22, S36/37, S45, S60. Thiodicarb is classified by WHO as 'moderately hazardous' and by the US EPA as 'slightly toxic' (Lewis *et al*., 2007).

An ADI of 0.01 mg/kg bw/day has been established for thiodicarb with a safety factor of 100 applied, based on NOAEL of 12.8 mg/kg bw/day from a one-year dog feeding study (Lewis *et al*., 2007).

5.2 Methomyl

The EC risk classification is: T+ – Very toxic: R28; N - Dangerous for the environment: R50, R53. The EC Safety classification is S1/2, S22, S36/37, S45, S60, S61. Methomyl is classified by WHO as 'highly hazardous' and by the US EPA as 'highly toxic' (Lewis *et al*., 2007).

An ADI of 0.0025 mg/kg bw/day has been established for methomyl (USEPA, 1998) using a SF of 100, based on a 2.6 mg/kg bw/day from a 2-year dog feeding study (Lewis *et al*., 2007).

6 References

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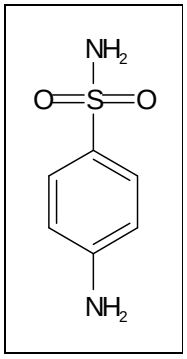
Appendix 12.44 Sulfanilamide

1 Introduction

Sulfanilamide (4-aminobenzenesulfonamide; CAS No. 63-74-1) is a metabolite of the herbicide Asulam (methyl [(4-aminophenyl)sulfonyl]carbamate; CAS No. 3337-71-1), that is formed within the surrounding soil.

The structure of sulfanilamide is presented in Figure 1.

Figure 1: Sulfanilamide



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Asulam

Asulam is a selective, post emergent systemic herbicide that is used primarily on sugarcane (95%); other applications include Christmas tree plantings, turf, ornamentals (juniper and yew) and non-crop lands (US EPA, 2002). Asulam is usually formulated and sold as the sodium salt (Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Asulam has high solubility in water (4000 mg/L) and, in assessing the sorption to soil, a Koc value of 130L kg-1 has been described, suggesting moderate mobility in the environment (Lewis et al., 2007).

Asulam is susceptible to degradation in soil under aerobic conditions, with a degradation half-life of 9 days under field conditions; asulam is not considered to be persistent (Lewis et al., 2007).

Sulfanilamide is a major metabolites formed through degradation of asulam, with an estimated maximum occurrence fraction of 0.139 (14%; Lewis et al., 2007).

2.2.2 Metabolite: Sulfanilamide

Sulfanilamide has high solubility in water (1000 mg/L) and, in assessing the sorption to soil, a Koc value of 145 L kg-1 has been described, suggesting moderate mobility in the environment (Lewis et al., 2007).

No further information relating to either the physical properties or environmental fate of the metabolite was found.

2.3 Potential routes of human exposure

Occupational exposure to asulam may occur through inhalation of dust particles and dermal contact with this herbicide during or after its application or at workplaces where asulam is produced or used. The general population may be exposed to asulam via dermal contact, inhalation of ambient air or through ingestion of drinking water and food containing the herbicide (US EPA, 2002).

Although no data on routes of exposure of humans to the metabolite sulfanilamide was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of asulam and when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics of parent and metabolite
No information is available on the toxicokinetics of asulam in humans.

Detailed reports of toxicokinetic studies for asulam available in the public domain were found to be limited. Metabolic studies have been carried out in rats administered either a single oral or intravenous dose of asulam. No localisation of compound in tissues was observed and asulam was excreted rapidly, predominantly via urine. The major excretory product was identified as unchanged parent, with acetylasulam and acetylsulphanilamide as minor metabolites (US EPA, 2002).

No information on the toxicokinetics of sulfanilamide was identified.

4 Toxicity Profile
4.1 Asulam
4.1.1 Acute Toxicity
No information is available on the acute toxicity of asulam in humans.

Asulam is a mild irritant to the eye but not the skin; no evidence of skin sensitisation has been found (US EPA, 2002). Experimentally, asulam shows low acute toxicity in rats, with LD50's of 5000 mg/kg, 10,000 mg/kg and 1.8 mg/m3 when given by the oral, dermal or inhalation routes, respectively (Lewis et al., 2007).

4.1.2 Repeat dose toxicity
No information on the repeat dose toxicity of asulam in humans was identified.

Experimentally, chronic exposure effects were assessed in a 6-month feeding study in which dogs were administered asulam in the diet. Treatment related findings, apparent in the high-dose group, were reported as; reduced body weight gain and food consumption in males and females; increased frequency of emesis and diarrhoea in both sexes; increased absolute thyroid weights in males and females at mid-and high doses; increased relative kidney weights; decreased absolute and relative testes weight; decreased absolute lung weight. Plasma and brain cholinesterase activities were not affected by asulam in either males or females (US EPA, 2002).

A chronic toxicity study in rats also demonstrated that the target organ of asulum toxicity is the thyroid. In a 2-yar feeding study, hyperplastic changes in thyroid follicular cells were observed in male rats at levels ≥ 180 mg/kg/day and a NOAEL of 36 mg/kg/day was established (US EPA, 2002).Other toxicological effects included adrenal medullary hyperplastic changes in males and decreased body weight gains in both sexes.

4.1.3 Carcinogenicity and genotoxicity

No information relating to carcinogenic or genotoxic effects following repeated exposure of humans to asulum was found.

In the chronic feeding study in rats described above, administration of asulum was associated with a statistically significant increase in thyroid gland C-cell carcinomas in low and mid-dose males. A statistically significant increase in incidence of adrenal medullary pheochromocytomas was also seen in males receiving the highest dose of asulam (US EPA, 2002). Asulam is classified as a possible human carcinogen.

The mutagenic and genotoxic potential of asulam has been assessed in a number of in vitro and in vivo assays; data indicated no mutagenic / genetic toxicity concern (US EPA, 2002).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of asulam in humans was identified.

In a 2-generation reproductive toxicity study, Charles River CD rats were administered asulam in the diet at levels up to 25,000 ppm (equivalent to 1250 mg/kg/day). Toxicity was apparent in F1 female organ weights, with a significant decrease in mean ovarian weight and absolute liver weight at 25,000 ppm and decreased mean relative weight at ≥ 1000 ppm. Fewer live births per litter were noted at 5000 and 25,000 ppm and F1 parents showed a lower fertility index at levels ≥ 5000 ppm. A reproductive NOEL of 1000 ppm (250 mg/kg/day) was established (US EPA, 2002).

Developmental toxicity of asulam has been evaluated in the rat and the rabbit. In rabbits, no treatment-related effects on developmental parameters were seen at levels up to 750 mg/kg/day. In rats, an increase in pre-implantation loss was observed at doses of 1500 mg/kg/day. A developmental NOEL of 300 mg/kg/day has been established (US EPA, 2002).

4.2 Sulfanilamide

4.2.1 Acute Toxicity

No information on the acute toxicity of sulfanilamide to humans was identified.
Experimentally, sulfanilamide shows low acute toxicity in rats, with an LD50 of 3900 mg/kg when given by the oral route (Lewis et al., 2007).

4.2.2 Repeat dose toxicity

No information on the repeat dose toxicity of sulfanilamide in humans was identified.
Searches were made for publicly available information on the toxicity of sulfanilamide, and by use of the online programme ChemIDPlus (US NLM, 2003). However, the toxicity database identified was extremely limited. Additional information was, therefore, sought through application of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Summaries of the toxicity data predicted for sulfanilamide from these tools are summarised below (Table 1).

DEREK identified a number of structural alerts and the following conclusions were drawn:

- it is considered Equivocal (i.e. there is uncertain evidence) that sulfanilamide will cause bladder urothelial hyperplasia in humans. This endpoint is predicted because sulfanilamide is an aryl sulphonamide.

- it is considered Certain (i.e. there is evidence) that sulfanilamide will be cause hepatotoxicity in humans. This endpoint is predicted because sulfanilamide is a 4-Aminophenylsulphonamide or 4-aminophenylsulphone

- it is considered Certain (i.e. there is evidence) that sulfanilamide will be cause phototoxicity in humans. This endpoint is predicted because Sulfanilamide is an aryl sulphonamide.

- it is considered Certain (i.e. there is evidence) that sulfanilamide will be cause ocular toxicity in humans. This endpoint is predicted because Sulfanilamide is an Aryl sulphonamide.

- it is considered plausible (i.e. there is a weight of evidence) that sulfanilamide will cause skin sensitisation in humans. This endpoint is predicted because sulfanilamide is an aromatic primary or secondary amine.

- it is considered plausible (i.e. there is a weight of evidence) that sulfanilamide will cause thyroid toxicity in humans. This endpoint is predicted because sulfanilamide is a 4-aminoaryl sulphonamide or precursor.

The analysis by TOPKAT suggested that sulfanilamide was:

- non-mutagenic; and
- not a developmental toxicant

Prediction of the carcinogenic potential of sulphanilamide using TOPKAT was not possible.

Table 1: Predicted toxicity data for sulfanilamide using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$ (range)				
Asulam (experimental data)	5000	1.8	NOAEL: 36 mg/kg/day (rats) Target Organ: Thyroid	Negative	Thyroid Carcinomas (rats and dogs)	Negative
Sulfanilamide (TOPKAT)	3700 (796.1-10,000)	4800 (636.5-10,000)	MTD (feed/drink) 77 mg/kg MTD (oral gavage) 77 mg/kg	Negative	Indeterminate	Negative
Sulfanilamide (DEREK)	n/a	n/a	In humans, *Equivocal* for bladder urothelial hyperplasia; *Certain* for hepatoto-xicity, photo-toxicity, ocular toxicity; *Plausible* for skin sensitisation and thyroid toxicity	No alert	Equivocal in humans	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software;

5 Guidelines and Standards

5.1 Asulam

No ADI for asulam could be found, however an ADI of 0.36 mg/kg bw/day has been established using a safety factor (SF) of 100 , based on a NOAEL of 36 mg/kg bw/day from a 2-year rat feeding study with asulam sodium (Lewis *et al*., 2007).

Asulam is not listed under EC risk or safety classifications. Asulum is classified by WHO as 'unlikely to present acute hazard in normal use' and by the US EPA as 'not acutely toxic' (Lewis *et al*., 2007).

5.2 Sulfanilamide

No ADI has been proposed by any authoritative organisation for sulfanilamide. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

Overall, the toxicity profile for sulfanilamide is predicted to be similar to that of the parent, with the highest oral MTD predicted by TOPKAT for sulfanilamide being 77 mg/kg bw/day (regardless of route of administration). Applying a SF of 100 would give a nominal value of 0.77 mg/kg bw/day which would be higher than the

established ADI of 0.36 mg/kg bw/day for asulam sodium. Given this, it is – on a highly precautionary basis – therefore proposed to adopt a PSDV of 0.36 mg/kg bw/d for sulfanilamide, i.e. the same value as for the parent. This would give an additional safety factor of 200.

6 References

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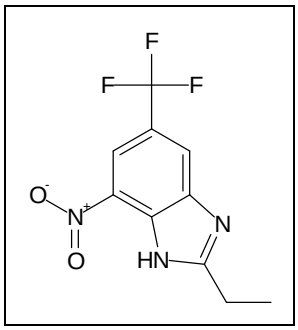
Appendix 12.45 2-Ethyl-7-nitro-5-(trifluoromethyl) benzimidazole

1 Introduction

2-Ethyl-7-nitro-5-(trifluoromethyl) benzimidazole is a metabolite of the herbicide trifluralin (2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine; CAS No. 1582-09-8).

The structure of 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole is presented in Figure 1.

Figure 1: Structure of 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Trifluralin

Trifluralin is a pre-emergence soil-incorporated herbicide for control of annual grasses and broadleaf weeds in crops including soybeans (52%), cotton (40%), vegetables, fruit and nuts (HSDB, 2002).

Trifluralin is sold worldwide under the commercial names Alpha Trifluralin 48EC, Ardent, Fargro Axit, Treflan and Uranus and is usually supplied as an emulsifiable concentrate that is mixed with water and used as a spray (Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Trifluralin has low solubility in water (0.221 mg/L) and, in assessing the sorption to soil, a Koc value of 8765 L kg-1 has been described suggesting no mobility in the environment (Lewis et al., 2007).

Trifluralin is slowly degradation in soil under aerobic conditions with degradation half-lives of 181and 170 days under laboratory (20oC) and field conditions respectively. Trifluralin is considered to be persistent (Lewis et al., 2007).

2.2.2 Metabolite: 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole

2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole is a metabolite formed through environmental degradation, and to a more limited extent metabolism of trifluralin.

No information relating to either the physicochemical properties or environmental fate of 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole was found.

2.3 Potential routes of human exposure

Occupational exposure to trifluralin may occur by inhalation or dermal contact during its production, formulation or application. Dermal exposure to trifluralin by farmworkers may also occur long after initial exposure as this compound has been found to be present in clothing used during application even after numerous washings (Stone & Stahr, 1989; Rigakis et al., 1987). The general population may be exposed to trifluralin via dermal contact or inhalation of ambient air or through ingestion of drinking water and food containing the herbicide (US EPA, 2002).

Although no data on routes of exposure of humans to the metabolite 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of trifluralin and when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics of parent and metabolite

No information on the toxicokinetics of trifluralin in humans was identified.

Published experimental data on the toxicokinetics of trifluralin was limited. Metabolic studies in rats and dogs have shown that trifluralin is not readily absorbed into the bloodstream from the GI tract. Approximately 80% of single oral doses in rats and dogs were excreted in faeces; the remainder was eliminated in urine (HSDB, 2002). Absorbed trifluralin is extensively metabolised; N-dealkylation and nitro-reduction are the principal pathways (Aizawa, 1982).

No information on the toxicokinetics of 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole was identified.

4 Toxicity Profile

4.1 Trifluralin

4.1.1 Acute Toxicity

Inhalation of trifluralin may cause irritation of the lining of the mouth, throat and lungs in humans. Skin sensitisation is considered possible in some individuals (EXTOXNET, 1996). However, no other information is available on other aspects of its acute toxicity in humans.

Trifluralin has low acute toxicity in rats; LD50's are 5000 mg/kg, 2000 mg/kg and 1.252 mg/m3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007). Unlike the situation for humans, experimentally trifluralin is not irritant to the eye or the skin of animals and no evidence of skin sensitisation has been found (HSDB, 2002).

4.1.2 Repeat dose toxicity

Repeated dermal exposure to trifluralin has been associated with development of contact dermatitis in humans (EXTOXNET, 1996).

In a two-year feeding study in Harlan rats fed diet containing trifluralin at up to 20,000 ppm, significant growth retardation and bile duct proliferation were observed at the highest dose; no other treatment effects were reported (NRC, 1977).

In a three-year feeding study in Beagle dogs at up to 25 mg/kg bw/day, increased bodyweight-relative liver weights were noted at the highest dose. Other effects included decreased red blood cell counts and increased methemoglobin, total serum lipids, triglycerides and cholesterol. A NOAEL of 10 mg/kg bw/day was established

(NRC, 1977). Trifluralin has also been reported been shown to cause liver and kidney damage after chronic oral exposure in other studies (US EPA, 1989).

4.1.3 Carcinogenicity and genotoxicity
No information on the carcinogenic or genotoxic potential of trifluralin in humans was identified.

Experimentally, Osbourne-Mendel rats were fed diets containing a low (4125 and 3744 ppm in males and females respectively) or high (8000 and 7917 ppm in males and females respectively) dose of trifluralin for 78 weeks, followed by a further (untreated) observation period of 33 weeks. No evidence of carcinogenicity was seen. In the same study, B6C3F1 mice were also given trifluralin in the diet at 3744 and 2000 ppm in males and 5192 and 2740 ppm in females for 78 weeks (again with a further observation period of 12 weeks). In females, a dose-dependent increase in incidence of hepatocellular carcinoma and alveolar/bronchiolar adenoma was reported. Squamous cell carcinoma of the stomach was also observed in treated females. No evidence of carcinogenicity was noted in the male mice (NCI, 1978).

Trifluralin was negative for mutagenicity in in vivo and bacterial or mammalian cell assays (EXTOXNET, 1996).

4.1.4 Reproductive and development toxicity
No information on the reproductive and developmental toxicity of trifluralin in humans was identified.

In a four-generation study in rats fed diets containing trifluralin at up to 10 mg/kg bw/day, reproductive capacity was unaffected (EXTOXNET, 1996).

In pregnant rabbits administered trifluralin at 224 or 500 mg/kg bw/day on gestation days 6 - 18, loss of appetite and body weight were reported in the dams. Fetal weight was decreased and there was an increase in the number of runts per litter at the highest dose (EXTOXNET, 1996).

In pregnant rats and rabbits orally dosed by gavage at up to 1000 mg/kg bw on gestation days 6 - 15 or 800 mg/kg bw on days 6 – 18, in rats and rabbits respectively, signs of maternal toxicity were observed. In rats, decreased body weight gain and food consumption were noted at 475 and 1000 mg/kg trifluralin while in rabbits abortions and/or deaths and decreased body weight gain and food consumption were noted at 225, 500 and 800 mg/kg bw/day. In rats, fetal viability and morphology were unaffected by treatment but, at the highest dose, fetal weights were depressed. In rabbits, at 500 and 800 mg/kg bw, decreased fetal viability and weight were noted but fetal morphology was unaffected. A NOEL of 225 mg/kg bw/day was established for maternal toxicity and 475 mg/kg bw/day for developmental toxicity in the rat while a NOEL of 100 mg/kg bw/day for maternal toxicity and 225 mg/kg bw/day for developmental toxicity were noted in the rabbit. Based on the findings of this study, trifluralin is not considered to be teratogenic (HSDB, 2002).

4.2 2-Eethyl-7-nitro-5-(trifluoromethyl) benzimidazole
Searches were made for published information on the toxicity of 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole in humans or animals, including use of the online programme ChemIDPlus (US NLM, 2003). However, No relevant information was identified.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK identified several structural alerts and the following conclusions were drawn:

- it is considered Plausible (i.e. there is a weight of evidence) that 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole will cause hepatotoxicity in humans. This endpoint is predicted because 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole is an aromatic nitro compound.

- it is considered Open (i.e. there is no overall weight of evidence) that 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole will be mutagenic in humans. This endpoint is predicted because 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole is an aromatic nitro compound.

- it is considered Plausible (i.e. there is a weight of evidence) that 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole will be carcinogenic in humans. This endpoint is predicted because 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole is an aromatic nitro compound.

The analysis by TOPKAT suggested that 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole was:

- a carcinogen; but
- not a developmental toxicant

Predictions of the reproductive and developmental toxicity of 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole using TOPKAT was not possible.

Table 1: Predicted toxicity data for 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Trifluralin (experimental data)	5000	1.252	NOAEL: 10 mg/kg/day (dogs) Target organs: liver and kidney	Negative	Hepatocellular carcinomas; alveolar/bronchio-lar adenomas; squamous cell carcinomas of the stomach (female mice)	Foetal toxicant
2-Ethyl-7-nitro-5-(trifluoromet-hyl) benzimid-azole (TOPKAT)	48.2 (7.7-300.7)	10,000 (10,000-10,000)	MTD (feed/drink) 264.6 mg/kg MTD (oral gavage) 732.5 mg/kg	UE	Positive	Negative
2-Ethyl-7-nitro-5-(trifluoromet-hyl) benzimid-azole (DEREK)	n/a	n/a	*Plausible* for hepatotox-icity in humans;	*Open* for mutagenicity in humans	*Plausible* in humans	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software; UE- unreliable estimate

5 Guidelines and Standards

5.1 Trifluralin

The EC risk classification is: Carcinogen category 3: R40; Xi - Irritant: R43; N - Dangerous for the environment: R50, R53. The EC safety classification is: S2, S36/37, S46, S60, S61. Trifluralin is classified by WHO as 'unlikely to present acute hazard in normal use' and by the US EPA as 'not acutely toxic' (Lewis *et al*., 2007).

An ADI of 0.015 mg/kg bw/d has been established for trifluralin using a safety factor (SF) of 100, from a three-year dog feeding study; the reference study for this could not be identified (Lewis *et al*., 2007).

5.2 2-Ethyl-7-nitro-5-(trifluoromethyl) benzimidazole

No ADI has been proposed by any authoritative organisation for 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The predictive systems applied to the metabolite have identified a range of possible toxic properties which, in most cases, reflect those observed experimentally for the parent. The lowest predicted oral MTD for 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole is 264.6 mg/kg (assuming administration via food or drinking water) Applying a SF of 100 would give a nominal value of approximately 2.6 mg/kg bw/day

which is considerably higher than the established ADI of 0.015 mg/kg bw/day for trifluralin. In the light of the possible range of toxicities that the metabolite might possess, it is – on a highly precautionary basis – therefore proposed to adopt a PSDV of 0.015 mg/kg bw/day for 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole, i.e. the same value as for the parent. This would give an overall safety factor of > 10,000.

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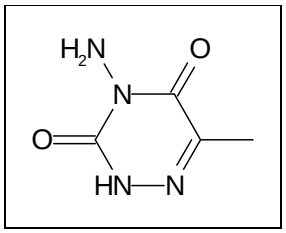
Appendix 12.46 CGA 294849

1 Introduction

CGA 294849 (CAS No. 1007-28-9) is a major metabolite of the insecticide pymetrozine (4,5-dihydro-6-methyl-4-[(*E*)-(3-pyridinylmethylene)amino]-1,2,4-triazin-3(2*H*)-one; CAS No. 123312-89-0) and is formed within the surrounding soil following application of the parent to plants. The metabolite is also formed during the metabolism of absorbed pymetrozine in humans.

The structure of CGA 294849 is presented in Figure 1.

Figure 1: Structure CGA 294849



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Pymetrozine

Pymetrozine is an insecticide used for control of aphids and whiteflies in vegetables, ornamentals, cotton, field crops, deciduous and citrus fruit; it is also used to control plant hoppers in rice (Tomlin, 1997).

The insecticide is sold worldwide under the commercial names Chess, Plenum, Endeavour and Fulfill; it is usually supplied as wettable granules that are mixed with water and used as a spray (Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Pymetrozine is moderately soluble in water (270 mg/L) and, in assessing the sorption to soil, a Koc value of 1510 L kg-1 has been described, suggesting slight mobility in the environment (Lewis et al., 2007).

Pymetrozine is moderately susceptible to degradation in soil under aerobic conditions, with degradation half-lives of 12.5 and 35.5 days under laboratory (20oC) and field conditions respectively; Pymetrozine is considered to be non-persistent (Lewis et al., 2007).

2.2.2 Metabolite: CGA 294849

CGA 294849 is one of the major metabolites formed following environmental degradation or metabolism of pymetrozine, with an estimates maximum occurrence fraction of 0.100 (10%; Lewis et al., 2007).

CGA 294849 is slightly susceptible to degradation in soil under aerobic conditions, with a degradation half-life 82.5 days under laboratory (20oC) conditions; CGA 294849 is considered to be moderately-persistent (Lewis et al., 2007).

No further information relating to either the physicochemcial properties or environmental fate of the metabolite was found.

2.3 Potential routes of human exposure

Occupational exposure to pymetrozine may occur by dermal contact with this compound at workplaces where pymetrozine is produced or during use. The general population may be exposed to pymetrozine by dermal contact, inhalation of ambient air or through ingestion of drinking water and food contaminated with the insecticide (HSDB, 2003).

Although no data on routes of exposure of humans to the metabolite CGA 294849 was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of pymetrozine and when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics of parent and metabolite

No information on the toxicokinetics of pymetrozine in humans was identified.

Toxicokinetic studies in rats given a single oral dose of pymetrozine at 0.5 or 100 mg/kg labelled on either the triazine or pyridine moiety. Absorption was rapid with maximum blood concentrations noted after 1 and 8 hours, for the low and high doses respectively. Overall absorption was lower at the high dose. Pymetrozine was distributed to tissues with the highest levels occurring in the kidney and liver. Pymetrozine was extensively metabolised (approximately 90 %) through oxidation reactions at the methyl substitute and the triazine-methylene group and cleavage reactions between the triazine and the pyridine ring systems (Okada, M, 1998). The calculated half-life for depletion of triazine residue n from all the tissues ranged from 2.9 - 4.8 hrs (low dose) or from 1.9 - 3.5 hrs (high dose) and, for pyridine residue depletion, was 31.7 - 110.3 hrs (low dose) and 2.5 - 13.9 hr (high dose). The pyridine residue metabolite is therefore more persistent than the triazine form (US EPA, 1999). Excretion of metabolites is predominantly via urine (56.3 - 80.3%) and faeces (15.4 - 38.9%; US EPA, 1999).

No information on the toxicokinetics of CGA 294849 was identified.

4 Toxicity Profile

4.1 Pymetrozine

4.1.1 Acute Toxicity

No information is available on the acute toxicity of pymetrozine in humans.
Pymetrozine is a mild ocular but not dermal irritant. There is some evidence of skin sensitisation (US EPA, 1999).

Experimentally, pymetrozine has low acute toxicity in rats; LD50's are 5820 mg/kg, 2000 mg/kg and 1.8 mg/m3 by the oral, dermal or inhalation routes respectively (Lewis et al., 2007).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of pymetrozine in humans was identified.
Experimentally, in a one year feeding study in rats fed treated diets containing pymetrozine at up to 3000 ppm (equivalent to 123.4 and 148.3 mg/kg bw/day for males and females respectively). Toxic effects included induction of hepatocellular hypertrophy at ≥ 100 ppm, reduced body weight and body weight gain at ≥ 1000 ppm, and increased bodyweight relative liver, spleen and kidney weights in males at ≥ 1000 ppm. In addition, relative weight of liver, spleen, kidney, brain and ovary in females and brain and testis of males were increased at 3000 ppm. Increased uterine dilation was also noted at this level. A NOAEL of 10 ppm (0.377 mg/kg bw/day) was established in males based on hepatocellular hypertrophy while that for females was

100 ppm (4.48 mg/kg/day) based on hepatocellular hypertrophy and reduced body weight and body weight gain (US EPA, 1999).

In a two-generation study in rats fed diets containing pymetrozine at up to 2000 ppm, systemic toxicity in parental animals was noted at the highest dose, comprising reduced body weight and body weight gain and associated reduction in food consumption. Systemic toxicity at 2000 ppm in F1 groups included reduced body weight body weight gain and food consumption. In F0 parents, hepatocellular hypertrophy was noted in males at 200 ppm and in both sexes at 2000 ppm. Hyperplasia of lymphatic follicles of splenic white pulp was also noted in F0 females given 2000 ppm. In F1 animals, hepatocellular hypertrophy was noted in males at 200 ppm and in both sexes at 2000 ppm while hypertrophy of the basophilic cells of the anterior pituitary (adenohypophysis) in males at 2000 ppm. Other findings included increased absolute and relative spleen and liver weights in F0 and F1 animals and decreased absolute and relative thymus weights in F1 animals at 2000 ppm. The liver was considered the target organ in both sexes in both generations. In addition, the spleen was a target organ in F0 females and the pituitary gland was affected in F1 males (US EPA, 1999).

A chronic feeding study in beagle dogs fed diet containing pymetrozine at up to 1000 ppm for one year showed toxic effects such as increased mean absolute and relative liver weight in males at 200 ppm. At the highest dose, mean absolute and relative liver weights were increased in both sexes and increased inflammatory cell infiltration in the liver and myopathy in the small and large intestine in males while two females showed anaemia. A NOAEL of 200 ppm (equivalent to 5.33 mg/kg bw/day) was established (US EPA, 1999).

4.1.3 Carcinogenicity and genotoxicity

No information on the carcinogenic or genotoxic potential of pymetrozine in humans was identified.

The one-year chronic feeding study in rats described above was extended to 2 years to assess carcinogenicity. Findings demonstrated the induction of liver tumours (benign hepatoma) at 1000 and 3000 ppm in females. However, due to the presence of hepatocellular hypertrophy at 1000 and 3000 ppm and a decreased body weight at 3000 ppm, it was considered possible that the maximum tolerated dosage had been exceeded (US EPA, 1999).

The mutagenicity of pymetrozine has been assessed in a reverse gene mutation assay in bacteria, and was found to be negative either with or without metabolic activation. Pymetrozine was negative in chromosome aberration and unscheduled DNA synthesis assays (US EPA, 1999).

4.1.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of pymetrozine in humans was identified.

In the two-generation study in rats fed diets containing pymetrozine at up to 2000 ppm, described above no reproductive parameters were affected by pymetrozine and a reproductive toxicity NOAEL of ≥ 2000 ppm (equivalent to 136.9 - 179.0 mg/kg bw/day for males and 151.6 - 186.5 mg/kg bw/day for females) was established (US EPA, 1999).

The potential developmental toxicity of pymetrozine was assessed in pregnant rats subject to oral gavage at 300 mg/kg bw/day from gestation days 6 - 15 inclusive. Signs of maternal systemic toxicity comprised reduced body weight gain at ≥ 100 mg/kg bw/day, and were associated with reduced food consumption. Developmental toxicity comprised increased skeletal abnormalities at 300 mg/kg/day including dumbbell-shaped thoracic vertebral centres, absent ossification of metatarsal #1, shortened rib #13, absent ossification of the proximal phalanx of anterior digit #5, absent ossification of the proximal phalanx of posterior digit #2, #3 and #4, and absent and poor ossification of the proximal phalanx of posterior digit #5. A developmental toxicity NOAEL of 100 mg/kg bw/day was established based on the increase in skeletal anomalies (US EPA, 1999).

4.2 CGA 294849

4.2.1 Acute Toxicity

No information on the acute toxicity of CGA 294849 in humans or animals was identified.

4.2.2 Repeat dose toxicity

No information on the repeat dose toxicity of CGA 294849 in humans was identified. Searches were made for published information on the toxicity of CGA 294849, and by use of the online programme ChemIDPlus (US NLM, 2003). However, No relevant information was identified.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK identified a number of structural alerts in the molecule and the following conclusions were drawn:

- it is considered plausible (i.e. there is a weight of evidence) that CGA 294849 will be cause hepatotoxicity in some animals (Dog, Guinea Pig, Hamster, Human, Mammal, Monkey, Mouse, Primate, Rabbit, Rat, Rodent). This endpoint is predicted because CGA 294849 is a hydrazine.

- it is considered plausible (i.e. there is a weight of evidence) that CGA 294849 will cause skin sensitisation in humans. This endpoint is predicted because CGA 294849 is a hydrazine or precursor.

- it is considered open (i.e. there is no overall weight of evidence) that CGA 294849 will be mutagenic in humans. This endpoint is predicted because CGA 294849 is an N-Amino heterocycle.

- it is considered plausible (i.e. there is a weight of evidence) that CGA 294849 will be carcinogenic in humans. This endpoint is predicted because CGA 294849 is a hydrazine.

- it is considered plausible (i.e. there is a weight of evidence) that CGA 294849 will be teratogenic in humans. This endpoint is predicted because CGA 294849 is a hydrazine.

The analysis by TOPKAT suggested that CGA 294849 was:

- mutagenic

Prediction of the carcinogenic potential and reproductive and developmental toxicity of CGA 294849 using TOPKAT was not possible.

Table 1: Predicted toxicity data for CGA 294849 using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/ H (range)				
Pymetrozine (experimental data)	5820	1.8	NOAEL: 5.33 mg/kg/day (dogs). Target organs: liver (both sexes), spleen (females) and pituitary (males)	Negative	Begnin hepatoma (rats)	Skeletal effects
CGA 294849 (TOPKAT)	5600 (950.2-10,000)	10,000 (8300-10,000)	MTD (feed/drink) UE MTD (oral gavage) UE	Mutagenic	UE	UE
CGA 294849 (DEREK)	n/a	n/a	*Plausible* for hepatotox-icity & skin sensitisation in humans	*Open* for mutagenicity in humans	*Plausible* in humans	*Plausible* for teratogenicity in humans

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/d – not determined; n/a – prediction not applicable to software; UE – unreliable estimate

5 Guidelines and Standards

5.1 Pymetrozine

The EC risk classification is: Carcinogen category 3: R40; N - Dangerous for the environment: R52, R53. The EC Safety classification is S2, S36/37, S61. Pymetrozine is classified by WHO as 'Slightly hazardous' and by the US EPA as 'Slightly toxic' (Lewis *et al*., 2007).

An ADI of 0.03 mg/kg bw/day has been established for pymetrozine, using a safety factor (SF) of 100, based on a NOAEL of 5.33 mg/kg bw/day from a one year dog feeding study reported above and applying a safety factor (SF) of 100 (Lewis *et al*., 2007).

5.2 CGA 294849

No ADI has been proposed by any authoritative organisation for CGA 294849 and the predictive systems used were unable to derive estimates of the MTD for this metabolite via the oral route making establishment of a 'project specific derived value' (PSDV) difficult. Nonetheless, in most respects it appears that the metabolite's toxicity profile may be similar to that of the parent, with structural alerts noted for carcinogenicity and developmental toxicity.

The main difference in toxicity appears to be in relation to concerns about the possible mutagenic potential of the CGA 294849. However, since the parent compound has been proven to be negative for this endpoint in a range of assays both in the absence and presence of metabolic activation, the validity of the predicted activity must be open to doubt. It is, therefore proposed for the purposes of risk assessment to establish a PSDV of 0.03 mg/kg bw/day based on the ADI of the parent compound; it should however be noted that there is some uncertainty with regards to the mutagenicity of the metabolite.

6 References

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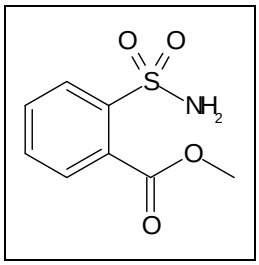
Appendix 12.47 Methyl 2-(aminosulfonyl)-benzoate

1 Introduction

Methyl 2-(aminosulfonyl)benzoate (IN-D5803) is a major degradation product of metsulfuron-methyl (CAS No. 74223-64-6) in soil. The structure of IN-D5803 is presented in Figure 1.

Figure 1: Structure of IN-D5803



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of metsulfuron-methyl

Metsulfuron-methyl is the BSI name for methyl 2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)benzoate (IUPIC). It is a herbicide used to control grasses and broad-leaved weeds mainly in cereals and land temporarily removed from production. As a systemic compound with foliar and soil activity, metsulfuron-methyl inhibits plant amino acid synthesis and therefore cell division in the shoots and roots of the plant. It is biologically active at low application rates and works rapidly after take up by the plant. Metsulfuron-methyl is also a predominant metabolite of iodosulfuron-methyl-sodium in soil and drinking water.

Metsulfuron-methyl is sold worldwide often under the product name Ally. It is often supplied as wettable granules and mixed with water to form a spray. The maximum application rate for metsulfuron-methyl is 6 g a.i/ha (PSD, 1995).

2.2 Environmental fate

2.2.1 Parent

Metsulfuron-methyl is highly water soluble (2790 mg/L at 20oC). In soil, the compound is broken down by chemical hydrolysis and microbial degradation. Half-life is typically 10 days under field conditions; breakdown is more rapid at lower soil pH or higher temperatures and at high levels of soil moisture. The major metabolites (fraction >10% of applied rate) are methyl 2-(aminosulfonyl)benzoate (Ref: IN-D5803), 2-(aminosulfonyl) benzoic acid (Ref: IN-D5119), phenylurea (Ref: IN-B5685), and saccharin (Ref: IN-00581).

The sorption Koc for metsulfuron-methyl was measured in the range of 4 – 60 ml/g, indicating very high mobility (EC, 2000).

2.2.2 Metabolite: IN-D5803

No information relating to either the physicochemical properties or environmental fate of IN-D5803 was identified.

2.3 Potential routes of human exposure

Exposure of humans to metsulfuron-methyl may occur through ingestion of contaminated food and water (oral route), or through occupational handling or by-stander exposure (inhalation or dermal contact).

Although no data on routes of exposure of humans to the metabolite IN-D5803 was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of metsulfuron-methyl, and, to a limited extent, when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics

No information is available on the toxicokinetics of metsulfuron-methyl in humans.

Total recovery of radiolabeled metsulfuron-methyl (dose unknown) administered to rats through the oral route was 91.6 - 103.8 %. The primary route of excretion was via the urine (approx. 71 - 95%) with faecal elimination being 4.8 - 13.3%. Overall, excretion was seen to almost complete within 48 hours. Elimination half-lives (males and females) were 13 - 16 hours for a single low dose, 9 - 12 hours when given as repeat dose over 21-days, and 23 - 29 hours for a single high dose. Tissue burdens were generally minimal (< 0.1% to 1%). Metabolic studies showed that the parent was recovered in both urine and faeces in all treatment groups and was the major contributor to the observed radioactivity (77 - 90% and 1.8 - 6.2% of administered dose, respectively). Four metabolites (saccharin and Metabolites I, II, and III) were found in both matrices and accounted for approximately 5.4 - 8.2% of dose. Two of the metabolites appear to result from sequential hydrolysis reactions terminating in the formation of saccharin, while one was formed by cleavage of the two ring structures (USEPA, 2002).

4 Toxicity Profile

4.1 Metsulfuron methyl

4.1.1 Acute Toxicity

No information on acute toxicity in humans has been identified.

In rats, metsulfuron-methyl has low acute toxicity; LD50 are > 5000 mg/kg bw, > 2000 mg/kg bw and > 5 mg/L for the oral, dermal and inhalation routes respectively. Metsulfuron-methyl did not cause irritation to the eyes of rabbits and did not cause skin sensitisation in Guinea pigs (EC, 2000).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of metsulfuron-methyl in humans was identified.

In a 90 day rat dietary study administering metsulfuron methyl, NOAELs of 68 mg/kg bw/day and 64 mg/kg bw/day for male and females were established. LOAELs of 521 mg/kg bw/day and 659 mg/kg bw/day were established for male and female rats, based on a transient decrease in body weight gain (EC, 2000; full study results unavailable).

In a dermal study in rabbits, a NOAL of 12 mg/kg bw/day and a LOAEL of 500 mg/kg bw/day were established, based on the development of diffuse/multifocal dermatitis. A NOAEL of 125 mg/kg bw/day and LOAEL of 500 mg/kg bw/day were identified for systemic toxicity based on an increased incidence of diarrhoea (EC, 2000; full study results unavailable).

4.1.3 Carcinogenicity and mutagenicity

No information was found on the carcinogenic and mutagenic effects of metsulfuron-methyl in humans.

In the 90 day dietary study described above, no evidence of carcinogenicity was found (EC, 2000; full study results unavailable).
All in vitro and in vivo mutagenicity tests produced negative results (EC, 2000; full study results unavailable).

4.1.4 Reproductive and development toxicity
No information on the reproductive and developmental effects of metsulfuron-methyl in humans was identified.

In a two-generation study in rats, parental systemic NOAELs were 34 mg/kg bw/day for males and 43 mg/kg bw/day for females, based on decreased pre-mating body weight gains by F0 rats in the highest dose group. The NOAELs for reproductive and developmental toxicity were 342 mg/kg bw/day for males and 475 mg/kg bw/day (USEPA, 2002; full study results unavailable).

In rabbits, a parental NOAEL of 25 mg/kg bw/day and a LOAEL of 1000 mg/kg bw/day were established based on increased mortality, decreased body weight gains, and clinical signs of anorexia, red or orange urine and /or exudate. Developmental NOAEL was 700 mg/kg bw/day. No developmental LOAEL was established (EC, 2000; USEPA, 2002; full study results unavailable).

4.2 IN-D5803
No information on the repeat dose toxicity of IN-D5119 in humans was identified.
Searches were made for published information on the toxicity of IN-D5803, including use of the online programme ChemIDPlus (http://chem2.sis.nlm.nih.gov/chemidplus/chemidlite.jsp). However, no relevant information was identified.

Additional information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK identified the presence of several structural alerts and the following conclusions were drawn:

- it is considered plausible (i.e. there is a weight of evidence) that IN-D5803 can cause bladder urothelial hyperplasia in humans. This endpoint is predicted because IN-D5803 is an aryl sulphonamide.

- it is considered plausible (i.e. there is a weight of evidence) that IN-D803 can cause ocular toxicity in humans. This endpoint is predicted because IN-D5803 is an aryl sulphonamide.

- it is considered plausible (i.e. there is a weight of evidence) that IN-D5803 can cause teratogenicity in humans. This endpoint is predicted because IN-D5803 is an aryl sulphonamide.

- it is considered plausible (i.e. there is a weight of evidence) that IN-D5803 can cause phototoxicity in humans. This endpoint is predicted because IN-D5803 is an aryl sulphonamide.

However, analysis by TOPKAT suggested that IN-D5803 is:

- non carcinogenic;
- non mutagenic; and
- not a developmental toxicant.

Table 1 Predicted toxicity data for IN-D5803 using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$ (range)				
Metsulfuron methyl (experimental data)	>5000	>5	NOAEL 64 mg/kg bw/d Main effect: Transient reduction in body weight gain	Negative	Negative	NOAEL Reproduction: 34 mg/kg/d Developmental: 475 mg/kg bw/d
IN-D5803 (TOPKAT)	10000 (8400-10000)	9600 (567.1-10000)	MTD (feed/drink) 1300 mg/kg MTD (oral gavage) 914.4 mg/kg	Negative	Negative	Indeterminate
IN-D5803 (DEREK)	n/a	n/a	Plausible for bladder urothelial hyperplasia and ocular toxicity in humans.	No alert	Plausible for bladder urothelial hyperplasia in humans	Plausible for teratogenicity in humans

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software

5 Guidelines and Standards

5.1 Metsulfuron-methyl

An ADI of 0.25 mg/kg bw/day has been established for metsulfuron methyl, based on a carcinogenicity study in rats in which NOAEL was 25 mg/kg bw/day, with a safety factor (SF) of 100 applied.

Metsulfuron-methyl is classified by EPA as Category III, and must bear the signal word "Caution" on commercial products. WHO classified metsulfuron-methyl as ‘unlikely to present acute hazard in normal use’. The EC risk classification is: N - Dangerous for the environment: R50, R53.

5.2 IN-D5803

Due to the lack of availability of experimental data, it has not been possible to establish a robust NOAEL for IN-D5803, and no ADI has been proposed by any authoritative organisation. Therefore, for the purposes of this study a ‘project specific derived value’ (PSDV) has been derived for use in the risk assessment.

Given that there is a degree of divergence as to the predictions of potential toxic activities of the metabolite (with DEREK suggesting, amongst other activities possibility of hyperplastic and teratogenic responses), a precautionary approach to establishing a PSDV is clearly warranted. However, in terms of both acute and repeat

dose toxicity, predictive tools suggest that the toxic profile of the metabolite may be less than that of the parent. In particular, the oral MTD predicted by TOPKAT for IN-D5803 is 1300 mg/kg bw/day (regardless of form of administration). Applying a SF of 100 would give a nominal value of 13 mg/kg bw/day, which would be significantly above the established ADI of 0.25 mg/kg bw/day for the parent metsulfuron-methyl. Given this, it is – on a highly precautionary basis – therefore proposed to adopt a PSDV of 0.25 mg/kg bw for IN-D5803 i.e. the same value as for the parent; this will provide an overall SF of 5200.

6 References

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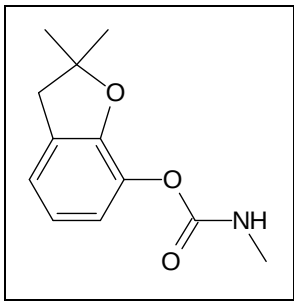
Appendix 12.48 Carbofuran

1 Introduction

Carbofuran (CAS No. 1563-66-2; IUPAC name: 2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate) is a major metabolite of the insecticide carbosulfan (CAS No. 55285-14-8) present in soil. It is also itself a broad spectrum carbamate pesticide for the control of insects, mites and nematodes on contact or after digestion. It is usually used against soil and foliar pests of field, fruit, and vegetable and forest crops (EXTOXNET, 1996).

Carbofuran is available in liquid and granular formulation, but the granule form is banned in the U.S. (EXTOXNET, 1996). It is not approved for use in the UK and majority of other European countries.

Figure 1: Structure of carbofuran



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of carbosulfan

Carbosulfan is registered in many countries, but not the UK, for use against a large number of plant pests on a wide range of crops. It acts by inhibiting the activity of acetylcholinesterase. It is usually supplied as dry granules applied directly to soil or seed bed (Lewis et al., 2007).

2.2 Environmental fate

2.2.1 Parent

Carbosulfan has a water solubility of 0.11 mg/L at 20oC. In the soil, the compound is degraded with a typical half-life of 21 days under field conditions; carbofuran is a major metabolite. The sorption Koc for carbosulfan is 9489 mg/g, indicating its immobile nature

2.2.2 Metabolite: Carbofuran

In soil, the half-life of carbofuran is 30 to 120 days. Chemical hydrolysis and microbial degradation appear to be important degradation processes. Chemical hydrolysis is expected to occur more rapidly in alkaline soil compared with neutral or acidic soils. It also breaks down in sunlight (USEPA, 2009b; EXTOXNET, 1996).

The sorption Koc for carbofuran is in the range 9.7 - 25.8 mg/g. Carbofuran is soluble in water (322 mg/L at 20oC) and is mobile to very mobile in sandy loam, silty clay and silty loam soils and moderately mobile in silty clay loam soils (EXTOXNET, 1996).

2.3 Potential routes of human exposure

The most likely route of exposure to carbosulfan or carbofuran is via oral, inhalation and skin contact in pesticide handlers, especially mixer/loaders who handle the concentrated material. Less often, people may be exposed from spray drift or from exposure to field residues. Exposure via oral consumption of contaminated food or drinking water is also possible.

3 Toxicokinetics

3.1 Carbosulfan

No information is available on the toxicokinetics of carbosulfan in humans.
Oral absorption in rats is very high; 90 - 98% recovery was noted after 168 hr following a single oral dose. The major route of excretion is urine although excretion also occurs via faeces and expired air. Less than 0.3% of administered dose was present in blood and tissue and < 2% in the carcass 168 hr following administration (IPCS, 2003).

In total, 10 metabolites have been identified in rats. Metabolites resulting from hydrolysis and oxidation were excreted mainly as sulfate/ glucuronide conjugates via urine. Compared with those given single doses, rats dosed repeatedly with carbosulfan at a low dose produced more 3-OH-7 phenol and 3-OH-carbofuran metabolites and less 3-keto-7-phenol and 7-phenol forms. This suggests repeated dosing may lead to metabolizing enzyme induction (IPCS, 2003).

In goats administered radiolabeled carbosulfan, the major route of elimination was shown to be urine with faeces and milk forming minor excretory routes. The concentrations of radiolabel in the blood increased over seven days following dosing. Some major metabolites (in conjugated, protein-bound or non-conjugated forms) present in the goats were the same as those seen in rats (IPCS, 2003).

3.2 Carbofuran

Following oral administration to mice and rats, carbofuran is rapidly absorbed, metabolized and eliminated, mainly in urine. After oral administration of [phenyl-14C]-carbofuran to rats, 92% of radiolabel was eliminated in urine and 3% in faeces. Most of the radiolabel was eliminated by 24 h after treatment. Identified metabolic pathways included hydroxylation, oxidation, hydrolysis and conjugation (IPCS, 1996).

4 Toxicity Profile

4.1 Carbosulfan

4.1.1 Acute Toxicity

No information on the acute toxic effects of carbosulfan in humans was identified.

Experimentally, information on the acute toxicity of carbosulfan relates to tests using formulations of carbosulfan that contained 25-50% active ingredient (a.i.).

In rats, the adjusted oral LD50s ranged from 90 to 250 mg carbosulfan/kg bw; LD50s tended to be lower for females than males. The inhalation LC50 was 0.61 mg carbosulfan/L. In rabbits, the adjusted dermal LD50 was > 2000 mg/kg bw. Clinical signs were generally suggestive of cholinesterase inhibition (IPCS, 2003; IUPAC, 2009).

In an acute neurotoxicity study in rats, a NOAEL of 0.5 mg/kg bw/day was established based on cholinesterase activity in brain tissue and erythrocytes four hours after dosing. No consistent differences in cholinesterase activity were observed on days 7 and 14 (IPCS, 2003).

Studies with formulations suggest that carbosulfan is mildly irritating to the eye and skin of rabbits. No evidence of dermal sensitization has been noted (IPCS, 2003; IUPAC, 2009).

4.1.2 Repeat dose toxicity

No information of the repeat dose toxicity of carbosulfan in humans was identified.

In a 21-day dermal toxicity study in rats, carbosulfan at 0, 5, 50 or 100 mg/kg bw/day did not produce any clinical signs of toxicity with the exception of a slight to moderate erythema and oedema at the highest dose. Body weight and food consumption were unaffected and no treatment-related haematological, biochemical or organ weight changes were noted. Other than dermal changes at the dose site, no histopathological changes were observed. However a statistically significant reduction in brain cholinesterase activity was found at 50 mg/kg bw/day. Based on this finding, a NOAEL of 5 mg/kg bw/day was established.

In a two-year feeding study, Charles River CD rats were administered carbosulfan at doses up to 2500 mg/kg diet, tremors, laboured breathing and eye-related changes were observed more frequently in animals fed carbosulfan at 500 or 2500 mg/kg diet. Significantly reduced mean body weight and food consumption were also seen at these two doses. Plasma, erythrocyte and brain cholinesterase activity was significantly decreased in males and females at a concentration of 500 or 2500 mg/kg. Treatment-related changes in the eye (focal iris atrophy, iris and absence of iris tissues) were also noted in both sexes at these doses while degenerative retinopathy was apparent only in females given 2500 mg/kg diet. A NOAEL of 20 mg/kg diet (equivalent to 1 mg/kg bw/day) was established based on the ocular toxicity, clinical signs and cholinesterase inhibition (IPCS, 2003).

In a two-year feeding study in mice, mean body weight and cholinesterase activity (plasma, erythrocyte and brain) were reduced at 500 and 2500 mg/kg diet. A NOAEL of 10 mg/kg diet (equivalent to 1.3 mg/kg bw/day) was established (IPCS, 2003).

4.1.3 Carcinogenicity and mutagenicity

No information on the carcinogenic or mutagenic potential of carbosulfan in humans was identified.

In the two-year feeding studies in rats and mice reported above, no evidence of carcinogenicity was found (IPCS, 2003).

A range of tests for genotoxicity in vitro and in vivo has been performed with carbosulfan and no evidence of genotoxic or mutagenic potential has been described (IPCS, 2003).

4.1.4 Reproductive and development toxicity

No information on the reproductive or developmental toxicity of carbosulfan in humans was identified.

In a three-generation study in Charles River CD rats, a diet containing carbosulfan at concentrations of 0, 10, 20 and 250 mg/kg (equivalent to 0, 0.67, 1.3 and 16.7 mg/kg bw per day) were administered for three generations. A NOAEL for parental toxicity of 20 mg/kg diet (equivalent to 1.3 mg/kg bw/day) was established based on decreases in body weight at 250 mg/kg. The NOAEL for reproductive toxicity was 250 mg/kg diet (equivalent to 16.7 mg/kg bw/day), the highest dose tested. However, the NOAEL for fetotoxicity was only 20 mg/kg (equivalent to 1.3 mg/kg bw/day)

because of reductions noted in litter size, pup weight and pup weight gain at 250 mg/kg.

4.2 Carbofuran

4.2.1 Acute toxicity

Carbofuran is highly toxic after acute oral administration. Carbofuran is an N-methyl carbamate (NMC) pesticide. As with other carbamates, carbofuran's cholinesterase-inhibiting effect is short-term and reversible. In humans, death may occur at high doses from respiratory system failure associated. Carbofuran has a steep dose-response curve and initial signs of toxicity include sweating, nausea, blurred vision (IPCS, 1996; HSDB, 2009).

Oral LD50 values range from 3 - 19 mg/kg bw in various species, and the inhalation LC50 is 0.08 mg/L. Dermal LD50 is > 1000 mg/kg (IPCS, 1996; HSDB, 2009).

Carbofuran caused no local irritation in rabbits after repeated dermal application for 7 or 21 days and is not a sensitizing agent in Guinea-pigs (IPCS, 1996)

4.2.2. Repeat dose toxicity

No information of the repeat dose toxicity of carbofuran in humans was identified.

In a four-week dietary study in dogs, 5 ppm (highest dose equivalent to 0.22 mg/kg bw/day) showed no effects on mortality, body weight, food consumption or plasma and erythrocyte cholinesterase activity; this was therefore considered to be the NOAEL.

In rabbits, dermal dosing with carbofuran as a 50% wettable powder at 0, 0.5, 1.0 or 2.0 mg/kg bw/day for 20 days resulted in dose-related deaths. Decreased body weight, inflammatory skin lesions and a decrease in general activity level were noted in all treated groups. Dermal lesions resolved within five days of cessation of treatment and no biochemical or histopathological changes were apparent. The NOAEL was < 0.5 mg/kg bw/day (HSDB, 2009).

In a 90-day gave study in rats at 0, 0.1, 0.3, 1.0 and 3.0 mg/kg bw/day, depression of erythrocyte and plasma cholinesterase activity was noted at the highest dose within one hour of administration when measured after three weeks of treatment; normal enzyme activity was apparent 24 hours after dosing. The NOAEL was considered to be 0.3 mg/kg bw/d (HSDB, 2009).

In two-year carcinogenicity and chronic toxicity studies, in mice, at dietary levels of 0, 20, 125, or 500 ppm and, in rats, at 0, 10, 20, or 100 ppm, NOAELs of 20 ppm (equivalent to 2.8 mg/kg bw/day) and 20 ppm (equivalent to 1 mg/kg bw per day) were established for in mice and rats respectively. These were based on inhibition of erythrocyte and brain acetylcholinesterase activity (IPCS, 2003).

4.2.3 Carcinogenicity and mutagenicity

Epidemiological studies in humans have found no association between carbofuran use and cancer risk (HSDB, 2009); carbofuran is classified by the US EPA as "not likely to be a human carcinogen" based on the absence of carcinogenicity in mice or rats (US EPA, 2009a; 2009b).

In the two-year studies, in mice and rats reported above, there was no evidence of any tumorigenic effect of treatment (IPCS, 2003).

Weak or no mutagenic effects have been reported in animals and bacteria (IPCS, 1996); carbofuran is considered to be non-mutagenic (IPCS).

4.2.4 Reproductive and development toxicity

In a three-generation rat study at dietary concentrations of 0, 20 or 100 ppm diet, reduced body weight gain was noted in parental animals and reduced growth and survival in pups were noted at the highest dose. The NOAEL was considered to be 20 ppm (equivalent to 1.6 mg/kg bw/day; IPCS, 1996).

In a reproductive toxicity study, adult male rats were fed carbofuran via their diet for 60 days. A NOAEL of 0.1 mg/kg body was determined based on testicular changes in parent males and maternal toxicity.

Female rats were administered carbofuran by gavage daily through pregnancy or during lactation days 0 to 21. Pups were weaned at day 21 and examined at day 90. The NOEAL was 0.2 mg/kg bw/day for male development toxicity on the basis of abnormality of sperm due to in utero exposure to carbofuran (HSDB, 2009).

In a further study, rats exposed to carbofuran through gestation day 1- 5 showed increased estrogenicity, pre-implantation loss and gestation length at > 0.4 mg/kg bw/day (HSDB, 2009).

In a teratogenicity study in rabbits orally dosed at 0, 0.2, 0.6 or 2 mg/kg bw, a NOAEL of 0.6 mg/kg bw/day was established for maternal toxicity (based on clinical signs) but no fetal effects were seen; NOAELs of 2 mg/kg bw/day for fetotoxicity and teratogenicity were proposed. In a further study in rabbits administered carbofuran at 0, 0.12, 0.5 or 2 mg/kg bw, a NOAEL of 0.5 mg/kg bw/day was found based on a slightly reduced body weight gain in dams and slightly increased skeletal variations in pups at 2 mg/kg bw/day. No evidence of teratogenicity was noted (IPCS, 1996).

Toxicity profiles for carbosulfan and carbofuran are summarised in Table 1 below:

Table 1: Summary of toxicity data for carbosulfan and carbofuran

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity/ Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg	LC50 $mg/m^3/H$				
Carbosulfan (experimental data)	90-250	0.61	NOAEL 1mg/kg/d Main effect: cholinesterase inhibition pathology of the eye.	Negative	Carcinogenicity potential not established	NOAEL Fetotoxicity: 1.3 mg/kg bw/d Reproductive toxicity: 16.7 mg/kg bw/d
Carbofuran (experimental data)	3-19	0.08	NOAEL 0.1 mg/kg/d Main effect: inhibition of brain and erythrocyte cholinesterase activity and body-weight reduction	Negative	Carcinogenicity potential not established	NOAEL Development: 0.2 mg/kg bw/d Reproductive toxicity: 0.1 mg/kg bw/d

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested.

5 Guidelines and Standards

5.1 Carbosulfan

The EC risk classification is: T+ - Very toxic: R26; T - Toxic: R25; Xi - Irritant: R43; N - Dangerous for the environment: R50, R53. Carbosulfan has been classified by WHO as “Moderately hazardous” (Lewis *et al.,* 2007).

A toxicologically-based ADI of up to 0.01 mg/kg bw/day was established for carbosulfan. This was based on a NOAEL of 1 mg/kg bw/day established for eye pathology, and inhibition of brain and erythrocyte cholinesterase activity and body-weight in a two-year rat study. A safety factor of 100 was used (IPCS, 2003).

5.2 Carbofuran

The EC risk classification is: T+ - Very toxic: R26/28; N - Dangerous for the environment: R50, R53. WHO has classified carbofuran as 'highly hazardous'. EPA classified carbofuran as Toxicity Category I, the most toxic category, based on its potency by the oral and inhalation exposure routes (Lewis *et al*., 2007).

A toxicologically-based ADI of up to 0.001 mg/kg bw/day for carbofuran was established based on a NOAEL of 0.1 mg/kg bw/day for male reproductive toxicity (testicular pathology) and maternal toxicity (abnormal behaviour) in a rat study using a 100-fold safety factor (Lewis *et al*., 2007); a value of 0.001 mg/kg bw/day is used for risk assessment.

The use of a short-term reproductive study was justified because it provided the most sensitive NOAEL from among the available toxicity tests. This ADI was noted to provide a SF of 3000 - 19000 compared with experimental acute oral toxicity for this compound. In considering this compound, the US EPA also applied a 500-fold factor which included a 5x allowance for child safety. Most recently, in 2008 the US EPA published a draft Notice of Intent to cancel all carbofuran registrations, based in part on that the acute dietary risks from carbofuran residues in food exceed the EPA’s level of concern (USEPA, 2009a).

6 References

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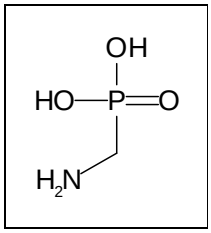
Appendix 12.49 Aminomethylphosphonic acid

1 Introduction

Aminomethylphosphonic acid (AMPA, CAS No 1066-51-9) is the primary degradation product of glyphosate (*N*-(phosphonomethyl)glycine; CAS No 1071-83-6) in plants, soil, and water.

The chemical structure of APMA is presented in Figure 1.

Figure 1 Structure of APMA



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of glyphosate

Glyphosate is a week organic acid used as a post-emergent, systemic and non-selective (or broad spectrum) herbicide to kill all plant types including grasses, perennials, and woody plants. It is used for both agricultural and non-agricultural purposes world-wide.

Glyphosate is mainly absorbed into the plant through leaves and then transported throughout the plant. The exact mode of action of glyphosate is unknown but it is thought that glyphosate inhibits elements of amino acid metabolism. This pathway of metabolism, known as the shikmic acid pathway, exists in higher plants and micro-organisms but not in animals.

Glyphosate is the world's most-used herbicide and the chemicals and biotechnology company Monsanto produces the market-leading form 'Roundup'. Glyphosate is usually formulated as the isopropylamine or trimethylsulfonium salt and supplied as a soluble concentrate that is mixed with water and applied as a spray. The application rates of glyphosate do not exceed 5.8 kg/ha (Buffin & Jewell, 2001).

2.2 Environmental fate

2.2.1 Parent

Glyphosate is highly water soluble (10500 mg/L at 20oC). The soil sorption Koc for glyphosate was measured in the range of 884 – 60000 ml/g, indicating that the chemical is immobile in soil. Metal complexes with humic acid in soil may be the main binding mechanism (EC, 2002).

In soil, the compound is rapidly broken down with a typical half-life of 12 days under field conditions; APMA is the major breakdown product. The degradation is pH sensitive (more rapid at lower soil pHs) and occurs more rapidly in aerobic than anaerobic conditions. A range of bacterial strains can degrade glyphosate, with the main route of biodegradation splitting of the C-N bond to produce AMPA (Buffin & Jewell, 2001).

2.2.2 Metabolite: AMPA

In soil, AMPA is persistent with typical half-life of 151 days under field conditions. The sorption Koc value for AMPA was 8027 ml/g, indicating its immobile nature (Lewis et al., 2007).

2.3 Potential routes of human exposure

Occupational workers and home gardeners may be exposed to glyphosate by inhalation and dermal contact during spraying, mixing, and cleanup. They may also be exposed by touching soil and plants to which glyphosate was applied. Occupationally, dermal exposure may also occur during glyphosate's manufacture, transport, storage, and disposal. For the general population, exposure is expected to occur through consumption of contaminated food and water.

Although no data on routes of exposure of humans to the metabolite AMPA was identified, it is plausible that exposure may occur as a result of its ingestion in water contaminated from the breakdown of glyphosphate or, to a limited extent when formed as a human metabolite following ingestion of the parent compound.

3 Toxicokinetics

Glyphosate is poorly absorbed via the gut and skin. After oral ingestion, 30 - 36% of glyphosate is absorbed in most test species. The highest tissue concentration, approximately 1% of oral dose, is located to bone. Biotransformation occurs to a very limited extent with the only metabolite AMPA accounting for only < 0.3% of the dose. The remainder is unchanged glyphosate. For absorbed glyphosate, 14 - 29% is excreted in urine and 0.2% or less in expired air. Biliary excretion, following intravenous dosing, was only 5 - 8%. In lactating goats, excretion in milk occurred to a minor extent (concentration < 0.1 mg/kg whole milk for a dose level of 120 mg/kg diet). Whole body clearance (99% of oral dose) occurs in approximately 168 h (IPCS, 1997).

AMPA is only moderately well absorbed from the gastrointestinal tract of rats with, for example, 13% of a radiolabeled gavage dose appearing in the urine within 12 hr, 18% by 24 hr and 20% by 120 hr. However, more than 50% of the administered radiolabel was excreted in faeces within 24 hr and 74% by 120 hr and while < 0.1% was recovered as expired CO2 within 24hr. After 120 hr, only 0.06% of radiolabel remained in the tissues with only minor residues present in the liver, kidneys and muscle (IPCS, 1997).

4 Toxicity Profile

4.1 Glyphosate

4.1.1 Acute Toxicity

In humans, deaths have occurred after ingestion of about 200 ml (three quarters of a cup) of glyphosate formulation (EC, 2002; HSDB, 2006).

In rats, glyphosate showed low acute toxicity; LD50s are > 2000 mg/kg bw for both the oral and dermal routes. In a four hour inhalation study in rats, the NOAEL was > 5 mg/L (EC 20002).

Glyphosate is a moderate to severe eye irritation but causes only slight dermal irritation. It is not a dermal sensitizer in Guinea pigs (EC, 2002; HSDB, 2006).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of glyphosphate in humans was identified.

In a 90-day feeding study, mice were given diets containing 0, 250, 500 or 2,500 mg/kg/ bw/day of glyphosate. Reduced body weight gain was found at the high dose; based on this finding, a NOEL for systemic toxicity was established as 500 mg/kg bw/day and a LOEL of 2500 mg/kg bw/day (HSDB, 2006).

In a 21-day dermal study in rabbits at 10, 1000 or 5000 mg/kg bw/day, effects were observed only at the highest dose; these included very slight erythema and oedema of intact and abraded skin and decreased serum lactic dehydrogenase activity in both sexes, and decreased food consumption in males. The NOAEL was 1000 mg/kg bw/day and the LOAEL was 5000 mg/kg bw/day (HSDB, 2006)

4.1.3 Carcinogenicity and mutagenicity

No information was found on the carcinogenic or mutagenic potential of glyphosate in humans.

In a 26-month feeding study in rats, glyphosate at the high dose (equivalent to 31.4 mg/kg bw/day for males and 34 mg/kg bw/day for females) did not show carcinogenicity or other toxic effects. The highest dose was therefore considered to be the NOAEL (HSDB, 2006).

Glyphosate is not mutagenic in a battery of in vitro and in vivo tests (HSDB, 2006).

4.1.4 Reproductive and development toxicity

In a two-generation feeding study in rats, effects were restricted to the high-dose group of 1500 mg/kg bw/day and included: frequent soft stools in the F0 and F1 males and females; decreased food consumption and body weight gain of the F0 and F1 males and females during the growth (pre-mating) period; and decreased body weight gain of the F1a, F2a and F2b male and female pups during the second and third weeks of lactation. Based on these findings, the systemic NOAEL and LOAEL were 500 mg/kg bw/day and 1500 mg/kg bw/day respectively. The reproductive NOAEL was 1500 mg/kg bw/day and developmental NOAEL and LOEL were 500 mg/kg bw/day and 1500 mg/kg bw/day respectively (HSDB, 2006).

4.2 AMPA

4.2.1 Acute Toxicity

No information is available on the acute toxicity of AMPA to humans.

AMPA did not induce dermal or ocular irritation in rabbits (HSDB, 2006).

4.1.2 Repeat dose toxicity

No information on the repeat dose toxicity of APMA in humans was identified.

In a 13-week study on rats (aged four weeks) fed a diet containing AMPA at levels designed to achieve 0, 400, 1200, or 4800 mg/kg bw/day, a NOAEL of 400 mg/kg bw/day was determined based on changes in body-weight, biochemistry and irritation of mucosal and submucosal layers of the urinary tract.

4.3 Carcinogenicity and mutagenicity

No information on the carcinogenic or mutagenic potential of APMA in humans or animals was identified.

4.4 Reproductive and development toxicity

No information on the reproductive and developmental toxicity of APMA in humans was identified.

In a range-finding study, groups of eight pregnant rats were given AMPA in corn oil at 0, 125, 250, 500, 750 or 1000 mg/kg bw/day by gavage on gestation days 6 - 15. The NOAEL for developmental toxicity was 500 mg/kg bw/day (IPCS, 1997).

Due to the limited nature of the toxicity data identified, additional insight was sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK identified one structural alert and the following conclusion was drawn:

- it is considered plausible (i.e. there is a weight of evidence) that AMPA may cause hepatotoxicity in some animals. This endpoint was predicted because AMPA is an organophosphorous di- or tri-ester.

The analysis by TOPKAT suggested that AMPA is:

- non-carcinogenic; and
- non-mutagenic

Prediction of the reproductive and developmental toxicity of AMPA using TOPKAT was not possible

Table 1: Experimental and Predicted toxicity data of glyphosate and AMPA

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H				
Glyphosate (experimental data)	>2000	>5	NOEAL 500 mg/kg bw/day	Negative	not established	NOAEL Reproduction: 1500 mg/kg/d Development: 500 mg/kg bw/d
AMPA (experimental data)	n/d	n/d	NOAEL 400 mg/kg bw/day	n/d	n/d	Developmental NOAEL: 500 mg/kg bw/day
AMPA (TOPKAT)	37 (2.8-486)	2000 (92.3-10,000)	MTD (feed/drink) 3.1 estimate MTD (oral gavage) 8.5 mg/kg	Negative	Negative	UE
AMPA (DEREK)	n/a	n/a	*Plausible for hepatotoxi-city*	No alert	No alert	No alert

LD_{50} : lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; - : no data; MTD – maximum tolerated dose; n/a – prediction not applicable to software; UE-unreliable estimate

5 Guidelines and Standards

5.1 Glyphosate

The EC risk classification is: [Xi - Irritant: R41], [N - Dangerous for the environment: R51, R53]. Glyphosate is categorised by WHO as “product unlikely to present acute hazard in normal use”. The US EPA ranks glyphosate in toxicity category III (these products bear the label: ‘Caution’; Lewis *et al*., 2007).

An ADI of 0.3 mg/kg bw/day has been established for glyphosate based on a 26 month carcinogenicity study in rats for which NOAELs of 31 and 34 mg/kg bw/day were established for males and females, respectively; a safety factor (SF) of 100 applied (Lewis *et al*., 2007).

At the joint meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group (Lyon 1997), it was concluded that AMPA was considered to be of no greater toxicological concern than its parent compound. This Group therefore established a group ADI for AMPA alone or in combination with glyphosate of up to 0-0.3 mg/kg bw/d, based on the 26-month study in rats. This was based on a safety factor of 100. An ADI of 0.3 has therefore been adopted for risk assessment of AMPA.

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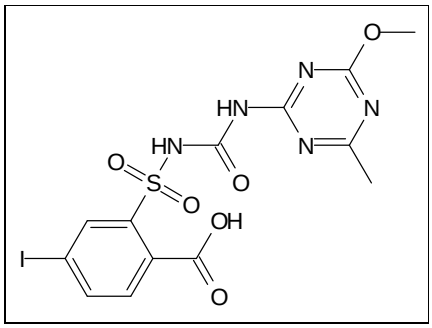
Appendix 12.50 AE F145740

1 Introduction

AE F145740 (4-iodo-2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)ureidosulfonyl] benzoic acid) is a minor metabolite of iodosulfuron methyl (CAS No. 185119-76-0) formed through the hydrolysis of the methylester (USEPA, 2002). The structure of AE F145740 is presented in Figure 1.

Figure 1: Structure of AE F145740



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of iodosulfuron methyl

Iodosulfuron methyl belongs to the general class of herbicides termed sulfonylureas. It is often supplied as the iodosulfuron methyl-sodium variant (CAS NO. 144550-36-7), the data used in this document were therefore derived from studies on iodosulfuron methyl-sodium rather than iodosulfuron methyl if not otherwise specified (EC, 2002; USEPA, 2002). Iodosulfuron-methyl-sodium selectively inhibits acetolactate synthase in susceptible species.

It is often sold as wettable granules and mixed with water to form a spray for post-emergence use to control grass and broad-leaved weeds in cereals. The maximum allowed application rate for iodosulfuron-methyl-sodium is 0.01 kg/ha (EC, 2003).

2.2 Environmental fate

2.2.1 Parent

Iodosulfuron-methyl-sodium is highly water soluble (25000 mg/L at 20oC). In the soil, the compound is degraded with a typical half life of 8 days under field conditions. The major metabolites (fraction >10% of applied rate) are AE F059411, AE F161778 and metsulfuron-methyl. The sorption Koc for iodosulfuron-methyl-sodium was measured in the range of 10 – 152 ml/g, indicating very high to high mobility (EC, 2003).

2.2.2 Metabolite: AE F145740

The sorption Koc value of AE F145740 in the soil is not known. No further information relating to either the physicochemcial properties or environmental fate of AE F145740 was identified.

2.3 Potential routes of human exposure

Exposure of humans to iodosulfuron methyl-sodium may occur through ingestion of contaminated food and water (oral route), or through occupational handling or by-stander exposure (inhalation or dermal contact).

Although no data on routes of exposure of humans to the metabolite AE F145740 was identified, it is plausible that exposure may occur as a result of its ingestion in food and water contaminated from the breakdown of iodosulfuron methyl-sodium (oral route) or when formed as a human metabolite from ingestion of the parent compound.

3 Toxicokinetics

No information on the toxicokinetics of iodosulfuron methyl in humans was identified.

In rats, iodosulfuron methyl was rapidly and highly absorbed after a single oral dose. Elimination of radioactivity occurred primarily in the urine (males 78.5%, females 85 %), mostly within 24 h of dosing, and was essentially complete within 3 days of dosing. The elimination also occurred in the faeces (males 19.2%, females 10.1%). Rats excreted majority of the dose as parent via the urine (48.7-86.3%) and faeces (1.1-11.1%). The major isolated metabolite was AE F145741. Minor routes metabolism for iodosulfuron-methyl included hydrolysis of the methylester to form 4-iodo-2-[3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)ureidosulfonyl]benzoic acid (AE F145740; 0.9-4.5% dose); O-demethylation of the triazine ring to form methyl 2-[3-(4-hydroxy-6-methyl-1,3,5-triazin-2-yl)ureidosulfonyl]-4-iodobenzoate (AE F148741; 1.5-8.2% dose); or hydroxylation of the methyl group on the triazine ring to form methyl 2-[3-(4-hydroxymethyl-6-methoxy-1,3,5-triazin-2-yl)ureidosulfonyl]-4-iodobenzoate (AE F168532; 0.3-6.6% dose). Each of these minor metabolites was present in both the urine and feces. By 3 days post-dose, <=5% of the dose remained in the blood and tissue of both sexes for both low and high dose groups. The total recovery of the administered dose was over 95 % for all groups, suggesting nearly complete absorption (USEPA). Dermal absorption in rats was less than 1% of the dose in 8 hours and up to 5% after 120 hours (NRA, 2001).

In dogs, 90-94% of oral dosed radioactivity was recovered within 72 h in both low and high dose groups. The elimination in urine and faeces accounted for 64-74% and 14-17% of applied dose. Majority of the dose was also excreted within 24 h as parent. The metabolites identified in the dogs were consistent with those in the rats.
No information on the toxicokinetics of AE F145740 was identified.

4 Toxicity Profile

4.1 Iodosulfuron methyl-sodium

4.1.1 Acute Toxicity

No information is available on the acute toxicity of iodosulfuron-methyl-sodium to humans.

In rats, iodosulfuron-methyl-sodium showed low acute toxicity, with oral LD50 2678 mg/kg bw, dermal LD50>2000 mg/kg bw and inhalation LC50 > 2.81 mg/l (EC, 2003). It was a moderate eye irritant but not a skin irritant in rabbits. In guinea pigs, it did not cause skin sensitisation (NRA, 2001).

4.1.2 Repeat dose toxicity

In a 90 day study of oral exposure to iodosulfuron-methyl-sodium, NOAELs of 67 mg/kg bw/day and 74 mg/kg/day were established for male and for female rats, and 8.1 mg/kg bw/day and 8.4 mg/kg bw/day for male and female dogs. The depression of body weight, haematotoxic (dog) and hepatotoxic (rats) effects were main critical observations when higher dose was applied (NRA, 2001).

4.1.3 Carcinogenicity and mutagenicity

No information was found relating to carcinogenic and mutagenic potential of iodosulfuron-methyl-sodium in humans.

In a 2-year feeding study in rats, iodosulfuron-methyl-sodium at 700 ppm caused body weight gain reduction. Based on this, a NOAEL of 70 ppm was established, equivalent to 3 mg/kg bw/day. No evidence was found relating to mutagenicity for iodosulfuron-methyl-sodium (NRA, 2001).

4.1.4 Reproductive and development toxicity

In a two-generation reproduction study in rats, NOAEL for iodosulfuron-methyl-sodium was 37 mg/kg bw/day for males and 52 mg/kg bw/day for females, based on the reduction in parental body weight, fetotoxicity and post-natal toxicity at higher dose. The fetotoxicity was indicated by increases in the number of pups born dead and associated decreases in the number of live born pups while the post natal toxicity was indicated by decreases in the survival of pups, especially during the first 4 days of lactation (NRA, 2001).

4.2 AE F145740

No information on the repeat dose toxicity of AE F145740 in humans was identified. Searches were made for published information on the toxicity of AE F145740, including use of the online programme ChemIDPlus (SLS NLM, 2003). However, no relevant information was identified.

Information was, therefore, sought through use of the 'in-silico' toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK identified the presence of a number of structural alerts and the following conclusions were drawn: in the molecule leading to the conclusions that it is considered plausible (i.e. there is a weight of evidence) that AE F145740 may cause:

- it is considered plausible (ie there is a weight of evidence) that AE F145740 will cause bladder urothelial hyperplasia in humans. This endpoint is predicted because AE F145740 is an aryl sulphonamide.

- it is considered plausible (ie there is a weight of evidence) that AE F145740 will cause thyroid toxicity in humans. This endpoint is predicted because AE F145740 is an aromatic iodo compound.

- it is considered plausible (ie there is a weight of evidence) that AE F145740 will cause skin sensitisation in humans. This endpoint is predicted because AE F145740 is an aryl sulphonamide.

- it is considered plausible (ie there is a weight of evidence) that AE F145740 will cause phototoxicity in humans. This endpoint is predicted because AE F145740 is an aryl sulphonamide.

The analysis by TOPKAT also suggested that AE F 145740 was:

- carcinogenic; but
- non-mutagenic; and
- a non developmental toxicant.

Table 1: Predicted toxicity data for AE F145740 using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 $mg/m^3/H$ (range)				
Iodosulfuron-methyl-sodium (experimental data)	2678	2.81	NOAEL 3 mg/kg/d Main effect: reduction in body weight gain	Negative	Negative	NOAEL 37 mg/kg/d for female; 52 mg/kg/d for male
AE F145740 (TOPKAT)	10000 (1700-10000)	10000 (3800-10000)	MTD (feed/drink) UE MTD (oral gavage) 74.2 mg/kg	UE	Weight of evidence that AE F14570 may exhibit carcinogenicity	Non – development toxicant
AE F145740 (DEREK)	n/a	n/a	No alert	No alert	Plausible	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software; UE – unreliable estimate

5 Guidelines and Standards

5.1 iodosulfuron methyl-sodium

The EC risk classification is: N - Dangerous for the environment: R50, R53. Iodosulfuron methyl-sodium has classified by WHO as 'unlikely to present acute hazard in normal use' (Lewis *et al*., 2007).

An ADI of 0.03 mg/kg/bw/d has been established for iodosulfuron methyl-sodium, based on a two-year oral dose study in rats in which NOAEL of 3 mg/kg bw/day was determined; a safety factor (SF) of 100 was applied (EC, 2003).

AE F145740

Due to the lack of availability of experimental data, it has not been possible to establish a robust NOAEL for AE F145740, and no ADI has been proposed by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

TOPKAT and Derek both highlight the possible carcinogenic potential of the metabolite, unlike the parent compound for which no evidence of carcinogenicity has been identified. A number of other potential toxicities are also predicted for the metabolite that do not appear to correlate with findings for the parent. However, both the acute and repeat dose effect levels predicted appear higher than that of the parent. Thus, the oral MTD predicted by TOPKAT for F145740 is 74.2 mg/kg bw/day for oral gavage. Applying a SF of 100 would give a nominal value of 0.74 mg/kg bw/day, which would be significantly above the established ADI of 0.03 mg/kg bw/day for the parent iodosulfuron methyl-sodium. Given these considerations, it is therefore

proposed to adopt a PSDV of 0.03 mg/kg bw/day for F145740, i.e. the same value as for the parent. This will provide an overall SF of over 2000, which is considered to provide an adequate margin of safety.

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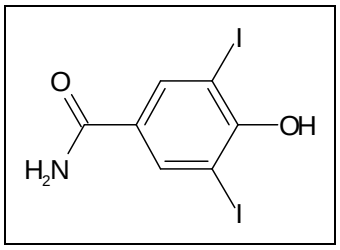
Appendix 12.51 3,5-Di-iodo-4-hydroxy-benzamide

1 Introduction

3,5-di-iodo-4-hydroxybenzamide is a major metabolite of the herbicide ioxynil (3,5-di-iodo-4-hydroxybenzonitrile; CAS No. 1689-83-4). It is formed during plant metabolism and within the surrounding soil (PSD, 1995). It is also formed during metabolism of absorbed ioxynil in humans and other organisms.

The structure of 3,5-di-iodo-4-hydroxybenzamide, is presented in Figure 1.

Figure 1: Structure of 3,5-di-iodo-4-hydroxybenzamide



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Ioxynil

Ioxynil is a selective contact benzonitrile herbicide that was first identified in 1963 and is currently sold worldwide under the commercial names Buctril, Totril, Briotril, Bantrol and Oxytril. Ioxynil is used to control broad leaf weeds in cereal crops and therefore has a large application area. Typical application rates for Ioxynil are quoted as 0.4 – 1.1 kg ha-1 (Miljøstyrelsen, 2003; Smith 1971). Esters of Ioxynil are also used in herbicide formulations, but at much lower concentrations than Ioxynil itself.

2.2 Environmental fate

2.2.1 Parent

Due to the presence of hydroxyl groups, ioxynil is water soluble (539 mg/L at pH 5 and 5.53 g/L at pH 9 at 20oC; EC, 2004). Studies assessing the sorption to soil of ioxynil are sparse, although a Koc value in the range 155 – 540L kg-1 has recently been described (Nielson et al., 2007) suggesting low to medium mobility (Bewick, 1994).

Ioxynil is susceptible to degradation through a variety of mechanisms (Millet et al., 1998; Nolte et al., 1995). Although degradation by photolysis immediately following application to leaf surface or soil may occur, biodegradation is the major pathway of elimination. Degradation studies have shown ioxynil to be readily degraded in soils (GraB et al., 2000; Hsu and Camper, 1975; Kjaer et al., 2003; Zaki et al., 1967); the half-life of ioxynil in soil has been estimated at less than one day (Kjaer et al., 2003), with near complete degradation within 19 days (Zaki et al., 1967). Despite a fairly rapid biodegradation, the half-life for mineralisation is approximated as 80 days (Hsu and Camper, 1975); this is suggestive of possible production of persistent metabolites.

2.2.2 Metabolite: 3,5-di-iodo-4-hydroxybenzamide

3,5-di-iodo-4-hydroxybenzamide is one of the major metabolites formed following hydrolysis of ioxynil, with an estimated maximum formation fraction of 0.105 (10.5%; Lewis et al., 2007).

A Koc value of 224 L kg-1 has been reported for 3,5-di-iodo-4-hydroxybenamide (Lewis et al., 2007) suggesting it to have low to medium mobility in the environment.

No further information on the physicochemical properties or environmental fate of 3,5-di-iodo-4-hydroxybenzamide was identified.

2.3 Potential routes of human exposure

Exposure of humans to the parent ioxynil may occur through ingestion of contaminated food and water (oral route), or through occupational handling or bystander exposure (inhalation or dermal contact; HSDB, 1996).

Exposure of humans to the metabolite 3,5-di-iodo-4-hydroxybenzamide may occur through ingestion of in food and water contaminated with ioxynil and its metabolites (oral route) or as a result of metabolism of absorbed ioxynil to the metabolite.

3 Toxicokinetics

No information on the toxicokinetics of ioxynil in humans was identified.

The toxicokinetics of ioxynil were established in a single oral dose study in which Sprague-Dawley rats were given 11 mg/kg bw of [14C]-ioxynil. In males approximately 85% of the dose was excreted in urine and 5% in faeces; the half-life was 28.3h. In females, approximately 77% and 8% of the dose was excreted in urine and faeces respectively, with a half-life of 40.7h (PSD 1995).

The same toxicokinetic study showed ioxynil to be widely distributed throughout the body within seven days of administration, with females having consistently higher tissue concentrations than males. Highest concentrations were found in plasma, and ioxynil does not have the potential to accumulate (PSD 1995).

Metabolic analysis, within the toxicokinetic study showed ioxynil to be metabolised, with unchanged ioxynil accounting for 26% of radiolabel in rat urine, with the remainder being associated with de-iodinated metabolites and conjugates (PSD 1995). Excretion of both the parent and its metabolites has been shown to be complete within 7 days, and to occur mainly via the urine (EC, 2004; HSDB, 1996).
No information on the toxicokinetics of 3,5-di-iodo-4-hydroxybenzamide was identified.

4 Toxicity Profile

4.1 Ioxynil

4.1.1 Acute Toxicity

Acute exposure of workers to ioxynil has been reported to cause excessive sweating, dizziness and headache (HSDB, 1996). Ingestion of 2–3 g in an adult human was fatal within 45 minutes (HSDB, 1996).

In rats, ioxynil shows moderate acute toxicity, with LD50’s of 110 mg/kg, 1050 mg/kg and 0.38 mg/m3 when given by the oral, dermal or inhalation routes, respectively (EC, 2004).

Ioxynil is an irritant to the eye, but not the skin. No evidence of skin sensitisation has been shown for ioxynil.

4.1.2 Repeat dose toxicity

The principal target organ of repeated exposure for ioxynil is the liver; effects also occur in the thyroid. Repeated exposure of humans to ioxynil has been associated

with reduced body weight gain, hypertrophy of the liver with enzyme induction, and thyroid hyperactivity (EC, 2004).

Experimentally, Fischer 344 rats fed for two years on diets containing between 0 and 500 ppm ioxynil showed increased liver (300 ppm) and kidney (80 and 300 ppm) weights, and a slight increase in thyroid weight at all doses. In a further two year rat study on Wistar rats given ioxynil at up to 100 ppm, a NOAEL of 0.5 mg/kg bw/day was established (PSD 1995; EC, 2004).

4.1.3 Carcinogenicity and mutagenicity

No information was found relating to carcinogenic and mutagenic effects in humans following exposure to Ioxynil.

Experimentally, no conclusive evidence of a genotoxic potential has been demonstrated for ioxynil. While there are some positive results using in vitro models, in vivo studies have been negative.

In animal studies, an increased incidence of thyroid follicular cell adenoma was seen in male and female Wistar rats fed a diet containing 100 ppm ioxynil for 2 years. An increased incidence of thyroid parafollicular cell tumours was also noted in females at 30 and 100 ppm. In contrast, mice receiving diets containing up to 100 ppm ioxynil for 18 months showed an increase in the incidence of hepatocellular carcinoma at 100 ppm (PSD 1995).

The relevance of these findings to humans exposed to ioxynil is not clear. Given the established marked susceptibility of the rat to disturbance of thyroid function and pathology consequent to altered hepatic metabolism, the changes in the liver may be of most significance to humans (PSD, 1995).

4.1.4 Reproductive and development toxicity

No information was found on the reproductive and developmental effects of ioxynil to humans.

Experimentally, maternally toxic doses (36 mg/kg bw/day) of ioxynil reduce litter size and pup weight in rats; a NOAEL of 2.5 mg/kg bw/day was established for reproductive effects (PSD 1995; EC, 2004). Other rat studies have found an increase in rates of malformation in rats, but only at a maternally toxic dose (36 mg/kg bw/day) and some evidence of growth retardation (reduced crown rump lengths) at levels (12 mg/kg bw/day) below the maternal toxic dose. Overall for rats, developmental NOAELs of 4 and 40 mg/kg bw/day have been established for oral and dermal routes respectively (PSD 1995; EC, 2004).

4.2 3,5-di-iodo-4-hydroxybenzamide

No information on acute toxicity of 3,5-di-iodo-4-hydroxybenzamide in humans or animals was identified.

Searches were made for published information on the toxicity of 3,5-di-iodo-4-hydroxybenzamide, including use of the online programme ChemIDPlus (US NLM, 2003). However, the information identified was extremely limited.

Additional information was, therefore, sought through use of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

DEREK identified the presence of one structural alert and the following conclusion was drawn:

- It is considered plausible (i.e. there is a weight of evidence) that 3,5-di-iodo-4-hydroxybenamide will exhibit thyroid toxicity in humans. This endpoint is predicted because 3,5-di-iodo-4-hydroxybenzoic acid is an aromatic iodo compound and also a polyhalogenated aromatic compound.

The analysis by TOPKAT suggested that 3,5-di-iodo-4-hydroxybenamide was:

- non-carcinogenic;
- non-mutagenic; but
- a developmental toxicant.

Table 1: Predicted toxicity data for 3,5-di-iodo-4-hydroxybenzamide using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/ H				
Ioxynil (experimental data)	110	0.38	NOAEL 0.5 mg/kg/d Main target organs: Liver and Thyroid	Genotoxic potential not established	Thyroid and liver carcinogen (rat)	Reduced litter size and pup weight (rats). Evidence of growth retardation (rats)
3,5-di-iodo-4-hydroxybenz-amide (TOPKAT)	22.1 (4.5 – 108.6)	10,000 (1500 – 10,000)	MTD (feed/drink) 4200 mg/kg MTD (oral gavage) 4200 mg/kg	Negative	Negative	Predicted developmental toxicant
3,5-di-iodo-4-hydroxyben-amide (DEREK)	n/a	n/a	Weight of evidence that 3,5-di-iodo-4-hydroxybenzoic acid will exhibit thyroid toxicity	No alert	Equivocal	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software

5 Guidelines and Standards

5.1 Ioxynil

An ADI of 0.005 mg/kg/bw/d has been established for Ioxynil, based on a two year rat study in which Wistar rats received ioxynil via the diet at levels up to 100 ppm (Defra PSD 1995; EC, 2004), with a safety factor (SF) of 100 applied (Lewis *et al*., 2007).

Ioxynil is classified by WHO as 'moderately hazardous', and by the US EPA as 'moderately toxic'. The EC risk classification is: Reproduction risk category 3: R63; Toxic: R23/25; Xn – Harmful: R21, R48/22; Xi – Irritant: R36; N – Dangerous for the environment: R50, R53

5.2 3,5-Di-iodo-4-hydroxybenamide

Due to the lack of availability of experimental data, it has not been possible to establish a robust NOAEL for 3,5-di-iodo-4-hydroxybenzamide, and no ADI has been published by an authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

Overall the metabolite is predicted to retain the thyrotoxic and developmental toxicant potential of the parent. In comparing repeat dose toxicity, the highest oral MTD predicted by TOPKAT for 3,5-di-iodo-4-hydroxybenzamide is 4200 mg/kg bw/day (irrespective of form of oral administration). Applying a SF of 100 would give a nominal value of 42 mg/kg bw/day, which would be significantly above the established ADI of 0.005 mg/kg bw/day for the parent ioxynil. Given this, it is – on a highly precautionary basis – therefore proposed to adopt a PSDV of 0.005 mg/kg bw/d for 3,5-di-iodo-4-hydroxybenzamide, i.e. the same value as for the parent; this will provide an overall SF of 8400. However, it should be borne in mind that

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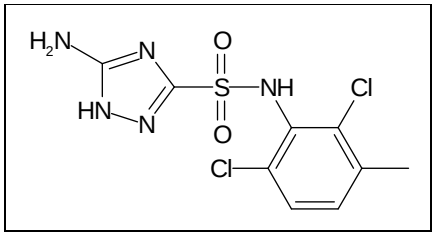
Appendix 12.52 5-Amino-*N*-(2,6-dichloro-3-methylphenyl)-1*H*-1,2,4-triazole-3-sulfonamide (ATSA)

1 Introduction

5-Amino-*N*-(2,6-dichloro-3-methylphenyl)-1*H*-1,2,4-triazole-3-sulfonamide is a major metabolite of the herbicide metosulam (CAS No. 139528-85-1). It is formed as a degradation product in the soil (PSD, 1996).

The structure of 5-amino-*N*-(2,6-dichloro-3-methylphenyl)-1*H*-1,2,4-triazole-3-sulfonamide is presented in Figure 1.

Figure 1: Structure of 5-amino-*N*-(2,6-dichloro-3-methylphenyl)-1*H*-1,2,4-triazole-3-sulfonamide



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of metosulam

Metosulam (CAS No. 139528-85-1) is the BSI name for 2',6'-dichloro-5,7-dimethoxy-3'-methyl[1,2,4]triazolo[1,5-a]pyrimidine-2-sulfonanilide (Lewis et al., 2007), which belongs to the triazolopyrimidine chemical group. Metosulam is selectively absorbed by roots and shoots of the plants and acts by the inhibition of acetolactate synthesis in susceptible species (PSD 9787/40,77). It is intended for use as a herbicide for the control of broad-leaved weeds in winter cereals. Metosulam is usually supplied as a suspension concentrate that is mixed with water and used as a spray. It is currently sold worldwide as the formulation EF 1077 which contains 100g /L metosulam. Typical application rate for metosulam is 10 g/ha (PSD, 1996).

2.2 Environmental fate

2.2.1 Parent

Metosulam is highly water soluble (700 mg/L at 20oC). In soil, the compound is degraded with a typical half life of 32 days under field conditions (http://sitem.herts.ac.uk/aeru/footprint/en/index.htm). The sorption Koc is in the range of 51 - 265 L/kg and is sensitive to pH (adsorption increases as soils pH decreases). According to the SSLRC classification, Metosulam is s mobile to moderately mobile and slightly to moderately persistent (PSD 1996).

5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide is one of the major metabolites formed following hydrolysis of metosulam with an estimated maximum formation fraction of 0.263 (26%; Lewis et al., 2007).

2.2.2 Metabolite: 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide

5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide is one of the major metabolites formed following hydrolysis of metosulam with an estimated maximum formation fraction of 0.263 (26%; Lewis et al., 2007).

In soil, 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide is degraded, with a half-life under laboratory conditions (20oC) of 37.2 days (Lewis et al., 2007).

A Koc value of 53 L/kg has been reported for 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide and the metabolite is considered to be mobile in the environment (Lewis et al., 2007).

2.3 Potential routes of human exposure

The exposure of humans to the parent compound metosulam may occur through ingestion of contaminated water and food (oral) or through occupational handling (dermal and inhalation).

Exposure to the metabolite 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide may occur through ingestion of contaminated food and water.

3 Toxicokinetics

No information relating to the toxicokinetics of metosulam in humans was identified.

Experimentally, interspecies and gender differences in the metabolism and toxicokinetics of Metosulam have been identified. In Sprague-Dawley rats, metosulam was absorbed following oral ingestion, with half lives of elimination via the urine or faeces of 29 - 37 h and 24 - 29 h in males, and 15 - 17h and 20 - 21 h in females, respectively. The mean total excreted via urine was 35.5% in males and 70.9% in females while that excreted via the faeces was 44.4% in males and 20.6% in females. Male rats were noted to excrete a large proportion of the absorbed dose as metabolites; these mainly comprised the 3-OH metosulam and 5-OH metosulam metabolites. In females excretion was mainly the parent compound. The total recovery of administered dose over 168 hrs was 84.5%-97.9% (PSD, 1996).

Persistence in the tissues was relatively low; the highest levels were in blood (0.12% of absorbed dose).

In dogs and mice, absorption was higher at low doses (5 mg/kg bw) than at a higher dose (100 kg/bw). Excretion was predominantly via the faeces and tissue persistence was again low (PSD, 1996).

Using in vitro rat and human skin models, dermal absorption appeared to be limited with more than 90 % of the applied dose recovered within 48 h (PSD 1996).

4 Toxicity Profile

4.1 Metosulam

4.1.1 Acute Toxicity

No information relating on the acute toxicity of metosulam to humans was identified.

In the rat, metosulam has low toxicity; LD50's of > 5000 mg/kg bw and > 1.9 mg/L have been reported for the oral and inhalation routes respectively. In mice, acute oral toxicity is also low (LD50 > 5000 mg/kg). The acute dermal toxicity of metosulam in rabbits is also low (LD50 > 2000 mg/kg bw). Overt signs of toxicity included diarrhoea and transient reduction in body weight gain (PSD, 1996).

In the rat, metosulam is a slight irritant to the eye but not the skin. No evidence of skin sensitisation has been reported (PSD, 1996).

4.1.2 Repeat dose toxicity
No information on the effects of repeated exposure to metosulam in humans has been identified.

In rodents, the main target organ is the kidney in rat and the liver in mice. Based on a 13 week repeated dose toxicity study in these species, NOAELs of 10 and 250 mg/kg bw/day has been established in the rat and mouse respectively (PSD, 1996).

4.1.3 Carcinogenicity and mutagenicity
No information on the carcinogenic or mutagenic potential of metosulam in humans was identified.

In a two-year feeding study in the rat, metosulam was not tumourigenic at 5 mg/kg bw/day but at higher doses, the incidence of renal tumours and nodular masses in the kidney and the incidence and severity of proximal tubule epithelial cell nuclear pleomorphism was increased compared with controls; a NOAEL for carcinogenicity of 5 mg/kg bw/day was established (PSD, 1996).

Standard mutagenicity studies were negative for metosulam (PSD, 1996).

4.1.4 Reproductive and development toxicity
No information was identified on the reproductive or developmental effects of metosulam in humans.

A rat multigeneration study established NOAELs for maternal reproductive toxicity and fetotoxicity of 30 and 100 mg/kg bw/day, respectively. At higher doses a reduction in kidney weight was noted (PSD, 1996).

4.2 5-Amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide
No information on the acute toxicity of 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide in humans was identified.

Searches were made for published information on the toxicity of 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide, including use of the online programme ChemIDPlus (SLS NLM, 2003). However, no relevant data was identified.

Additional information was, therefore, sought through use of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Results are summarised below (Table 1).

Table 1: Predicted toxicity data for 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Metosulam (experimental data)	5000	1.9	NOAEL 5 mg/kg/d Main target organ: kidney (rat)	Negative	Carcinogenicity potential not established	NOAEL 30 mg/kg/d (reproductive toxicity) 100 mg/kg/d fetotoxicity)
5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide (TOPKAT)	1800 (304-10,000)	10,000 (2000 – 10,000)	MTD (feed/drink) 304 mg/kg MTD (oral gavage) 844 mg/kg	Negative	Negative	Indeterminate
5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide (DEREK)	n/a	n/a	*Plausible* that for methaemoglobinaemia,ocular and thyroid toxicity in humans	No alert	*Equivocal* for carcinogenicity in humans	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD– maximum tolerated dose; n/a – prediction not applicable to software

DEREK identified the presence of a number of structural alerts and the following conclusions were drawn:

It is considered plausible (i.e. there is a weight of evidence) that 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide will exhibit carcinogenicity in humans. This endpoint is predicted because 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide is an aromatic iodo compound and also a polyhalogenated aromatic compound.

It is considered plausible (i.e. there is a weight of evidence) that 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide will exhibit thyroid toxicity in humans. This endpoint is predicted because 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide has structure similarity to 4-aminoaryl sulphonamide and precursor.

It is considered plausible (i.e. there is a weight of evidence) that 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide will exhibit ocular toxicity in humans. This endpoint is predicted because 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide has structure similarity to aryl sulphonamide

The analysis by TOPKAT suggested that 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide is:

non-carcinogenic; and
non-mutagenic.

Prediction of the reproductive and developmental toxicity of 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide using TOPKAT was not possible.

5 Guidelines and Standards

5.1 Metosulam

The EC risk classification is: Xn-Harmful: R48/22. Metosulam is classified by WHO as 'unlikely to present acute hazard in normal use' (Lewis *et al*., 2007).

An ADI of 0.005 mg/kg bw/day has been established for metosulam (PSD, 1996) based on a two-year dietary rat study in which rats received metosulam at up to 100 mg/kg bw/d .A safety factor (SF) of 100 was applied.

5.2 5-Amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide

Due to the lack of experimental data, it has not been possible to establish a robust NOAEL for 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide, and no ADI has been published by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

The highest oral MTD predicted by TOPKAT for 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide is 844 mg/kg bw (gavage administration). Applying a SF of 100 would give a nominal value of 8.44 mg/kg bw, which would be significantly above the established ADI of 0.005 mg/kg bw/day for the parent metosulam. It should be noted that the metabolite is predicted by TOPKAT to have a range of biological activities and given this, it is – on a highly precautionary basis – it is proposed to adopt a PSDV of 0.005 mg/kg bw/d for 5-amino-N-(2,6-dichloro-3-methylphenyl)-1H-1,2,4-triazole-3-sulfonamide, i.e. the same value as for the parent. This will provide an overall SF of 1688.

6 References

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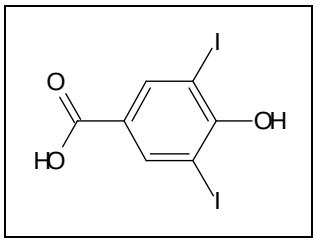
Appendix 11.53 3,5-Di-iodo-4-hydroxybenzoic acid

1 Introduction

3,5-Di-iodo-4-hydroxybenzoic acid (CAS No. 618-76-8) is a major metabolite of the herbicide Ioxynil (3,5-di-iodo-4-hydroxybenzonitrile; CAS No. 1689-83-4). It is formed during plant metabolism and in the surrounding soil (PSD, 1995). It is also formed during metabolism of absorbed ioxynil in humans and other organisms.

The structure of 3,5-di-iodo-4-hydroxybenzoic acid, is presented in Figure 1.

Figure 1: Structure of 3,5-di-iodo-4-hydroxybenzoic acid



2 Use and environmental fate of parent and potential human exposure routes

2.1 Use of Ioxynil

Ioxynil is a selective contact benzonitrile herbicide that was first identified in 1963 (Wain, 1963) and is currently sold worldwide under the commercial names Buctril, Totril, Briotril, Bantrol and Oxytril. Ioxynil is used to control broad leaf weeds in cereal crops and therefore has a large application area. Typical application rates for ioxynil are 0.4 – 1.1 kg ha-1 (Holtze et al., 2008; Smith 1971). Esters of ioxynil are also used in herbicide formulations but at much lower concentrations than ioxynil itself.

2.2 Environmental fate

2.2.1 Parent

Due to the presence of hydroxyl groups, ioxynil is water soluble (539 mg/L at pH 5 and 5.53 g/L at pH 9 at 20oC; EC, 2004). Studies assessing the sorption to soil of ioxynil are sparse although a Koc value of 155 – 540L kg-1 has been described suggesting low to medium mobility ((Nielson et al., 2007).

Ioxynil is susceptible to degradation through a variety of mechanisms (Millet et al., 1998; Nolte et al., 1995). Although degradation by photolysis immediately following application to leaf surface or soil may occur, biodegradation is the major pathway of elimination.

Degradation studies have shown Ioxynil to be readily degraded in soils (GraB et al., 2000; Hsu and Camper, 1975; Kjaer et al., 2003; Zaki et al., 1967); the half-life of ioxynil in soil is estimated at less than one day (Kjaer et al., 2003) with near complete degradation achieved by 19 days (Zaki et al., 1967). In contrast to the fairly rapid biodegradation, half-life of mineralisation is approximately 80 days (Hsu and Camper, 1975) which is suggestive of possible production of persistent metabolites.

2.2.2 Metabolite: 3,5-di-iodo-4-hydroxybenzoic acid

3,5-di-iodo-4-hydroxybenzoic acid is one of the major metabolites formed following hydrolysis of oxynil, with an estimated maximum formation fraction of 0.204 (20%; Lewis et al., 2007).

A Koc value of 323 l kg-1 has been reported for 3,5-di-iodo-4-hydroxybenzoic acid (Lewis et al., 2007), suggesting low to medium mobility in the environment.

No further information on the physicochemcial properties or environmental fate of 3,5-di-iodo-4-hydroxybenzoic acid was identified.

2.3 Potential routes of human exposure

Exposure of humans to the parent ioxynil may occur through ingestion of contaminated food and water or by inhalation or dermal contact during occupational handling or by-stander exposure (HSDB, 1996).

It is probable that exposure of humans to the metabolite 3,5-di-iodo-4-hydroxybenzoic acid may occur through ingestion of the metabolite in food and water contaminated with ioxynil and its metabolites (oral route) or as a result of direct metabolism following absorption of ioxynil.

3 Toxicokinetics

The toxicokinetics of ioxynil were established in an oral single dose study in which Sprague-Dawley rats were given 11 mg/kg bw of [14C]-ioxynil. In males approximately 85% of the dose was excreted in urine and 5% in faeces; the half-life was 28.3h. In females, approximately 77% and 8% of the dose was excreted in urine and faeces respectively; the half-life of elimination was 40.7 hr (PSD, 1995).

The same toxicokinetic study found ioxynil to be widely distributed throughout the body within seven days of dosing; females had consistently higher tissue concentrations than males. Highest levels were measured in plasma, and no evidence of accumulation in tissues was found (PSD, 1995).

Metabolic analysis in this study showed unchanged ioxynil to account for 26% of radiolabel in urine with the remainder being associated with de-iodinated metabolites and conjugates (PSD, 1995). Excretion of both the parent and its metabolites was complete within seven days, primarily via the urine (EC, 2004; HSDB, 1996).

No information on the toxicokinetics of the metabolite 3,5-di-iodo-4-hydroxybenzoic acid was identified.

4 Toxicity Profile

4.1 Ioxynil

4.1.1 Acute Toxicity

Acute exposure of workers to ioxynil has been reported to result in excessive sweating, dizziness and headache (HSDB, 1996). Ingestion of 2 – 3 g by an adult human has been fatal within 45 minutes (HSDB, 1996). In rats, ioxynil has moderate acute toxicity; LD50's are 110 mg/kg, 1050 mg/kg and 0.38 mg/m3 by the oral, dermal and inhalation routes respectively (EC, 2004).

Ioxynil is an irritant to the eye but not the skin. No evidence of skin sensitisation has been reported.

4.1.2 Repeat dose toxicity

The principal target organ following repeated exposure to ioxynil is the liver; effects also occur in the thyroid.

Repeated exposure of humans to ioxynil is associated with reduced body weight gain, hypertrophy of the liver (with enzyme induction) and thyroid hyperactivity (EC, 2004).

Experimentally in Fischer 344 rats fed for two years on diets containing up to 500 ppm ioxynil, increased liver (300 ppm) and kidney (80 and 300 ppm) weights and a slight increase in thyroid weight at all doses were noted. In a further two year rat study on Wistar rats given ioxynil at up to 100 ppm, a NOAEL of 0.5 mg/kg bw/day was established (PSD 1995; EC 2004).

4.1.3 Carcinogenicity and mutagenicity
No information on the carcinogenic and mutagenic effects of ioxynil in humans were identified

In animal studies, an increased incidence of thyroid follicular cell adenoma was seen in male and female Wistar rats fed diet containing 100 ppm Ioxynil for two years. An increased incidence of thyroid parafollicular cell tumours was also noted in females at 30 and 100 ppm. In mice receiving diets containing up to 100 ppm ioxynil for 18 months showed an increase in the incidence of hepatocellular carcinoma at 100 ppm. The relevance of these findings to humans exposed to ioxynil is not clear. Given the established marked susceptibility of the rat to disturbance of thyroid function and pathology consequent to altered hepatic metabolism, the changes in the liver may be of most significance to humans (PSD, 1995).

No conclusive evidence of a genotoxic potential has been demonstrated for ioxynil. While there are some positive results in in vitro models in vivo studies were negative.

4.1.4 Reproductive and development toxicity
No information was identified on the reproductive and developmental effects of ioxynil on humans.

Experimentally, maternally toxic doses (36 mg/kg bw/day) of ioxynil resulted in reduced litter size and pup weight in rats; a NOAEL of 2.5 mg/kg/d was established for reproductive effects (PSD 1995; EC, 2004).

Other studies have found an increase in rate of malformation in rats but again only at maternally toxic doses (36 mg/kg bw/day) and evidence of growth retardation (reduced crown rump lengths) at slightly lower doses (12 mg/kg bw) than that causing maternal toxicity. Overall, in rats a developmental NOAEL of 4 mg/kg/d mg/kg/day has been established (PSD 1995; EC, 2004).

4.2 3,5-di-iodo-4-hydroxybenzoic acid
No information on the acute toxicity of 3,5-di-iodo-4-hydroxybenzoic acid in humans was identified.

Searches were made for published information on the toxicity of 3,5-di-iodo-4-hydroxybenzoic acid, including use of the online programme ChemIDPlus (US NLM, 2003). However, no relevant information was identified.

Additional information was, therefore, sought through use of the ‘in-silico’ toxicity assessment systems DEREK (Deductive Estimation of Risk from Existing Knowledge, Lhasa Ltd.) and TOPKAT (Toxicity Prediction by Komputer Assisted Technology). Summaries of the toxicity data predicted for 3,5-di-iodo-4-hydroxybenzoic acid from these tools are summarised below (Table 1).

Table 1: Predicted toxicity data for 3,5-di-iodo-4-hydroxybenzoic acid using DEREK and TOPKAT software

Compound (data source)	Acute toxicity		Repeat dose toxicity	Genotoxicity / Mutagenicity	Carcinogenicity	Reproductive & Developmental toxicity
	LD50 mg/kg (range)	LC50 mg/m^3/H (range)				
Ioxynil (experimental data)	110	0.38	NOAEL 0.5 mg/kg/d Main target organs: Liver and Thyroid	Genotoxic potential not established	Thyroid and liver carcinogen (rat)	Reduced litter size and pup weight (rats). Evidence of growth retardation (rats)
3,5-di-iodo-4-hydroxybenzoic acid – (TOPKAT)	145.1 (30.2-698.1)	10,000 (10,000-10,000)	MTD (feed/drink) 1110 mg/kg MTD (oral gavage) 1110 mg/kg	Negative	Negative	Negative
3,5-di-iodo-4-hydroxybenzoic acid (DEREK)	n/a	n/a	Weight of evidence that 3,5-di-iodo-4-hydroxybenzoic acid will exhibit thyroid toxicity	No alert	Equivocal	No alert

LD_{50} – lethal oral dose for 50% of animals tested; LC_{50} – lethal inhalation dose for 50% of animals tested; MTD – maximum tolerated dose; n/a – prediction not applicable to software

DEREK identified the presence of structural alerts and the following conclusion was drawn:

It is considered plausible (i.e. there is a weight of evidence) that 3,5-di-iodo-4-hydroxybenzoic acid will exhibit thyroid toxicity in humans. This endpoint is predicted because 3,5-di-iodo-4-hydroxybenzoic acid is an aromatic iodo compound and also a polyhalogenated aromatic compound.

The analysis by TOPKAT suggested that 3,5-di-iodo-4-hydroxybenzoic was:

non-carcinogenic;
non-mutagenic; and
not a developmental toxicant.

5 Guidelines and Standards

5.1 Ioxynil

The EC risk classification is: Reproduction risk category 3: R63; Toxic: R23/25; Xn – Harmful: R21, R48/22; Xi – Irritant: R36; N – Dangerous for the environment: R50, R53. Ioxynil is classified by WHO as 'moderately hazardous', and by the US EPA as 'moderately toxic' (Lewis *et al*., 2007).

An ADI of 0.005 mg/kg bw/day has been established for ioxynil based on a two year dietary rat study at levels up to 100 ppm (PSD 1995; EU doc), with a safety factor (SF) of 100 applied (Lewis *et al*., 2007).

5.2 3,5-di-iodo-4-hydroxybenzoic acid

Due to the lack of availability of experimental data, it has not been possible to establish a robust NOAEL for 3,5-di-iodo-4-hydroxybenzoic acid, and no ADI has been published by any authoritative organisation. Therefore, for the purposes of this study a 'project specific derived value' (PSDV) has been derived for use in the risk assessment.

Although apparently likely to retain the thyrotoxic potential of the parent, the toxicity profile for 3,5-di-iodo-4-hydroxybenzoic acid is predicted overall to be somewhat less than that of the parent; the highest oral MTD predicted by TOPKAT for 3,5-di-iodo-4-hydroxybenzoic acid is 1100 mg/kg bw (irrespective of form of oral administration). Applying a SF of 100 would give a nominal value of 11 mg/kg bw which would be significantly above the established ADI of 0.005 mg/kg/bw for the parent ioxynil.
Given this, it is – on a highly precautionary basis – therefore proposed to adopt a PSDV of 0.005 mg/kg bw/d for 3,5-di-iodo-4-hydroxybenzoic acid (i.e. the same value as for the parent); this will provide an overall SF of 2200.

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Appendix 13 – Hazard Assessment Methodology and Literature Searches

Hazard assessments

Following agreement of the selected list of 53 pesticide metabolites, the most current review articles published by authoritative organisations, such as the World Health Organization/Food and Agriculture Organization (WHO/FAO) Joint Expert Committee on Food Additives (JECFA), Joint Meeting on Pesticide Residues (JMPR), Hazardous Substances Data Bank (HSDB), United States Environmental Protection Agency (US EPA); Pesticide Safety Directorate (PSD), European Commission (EC), Pesticide Properties Database (PPDB) and International Agency for Research on Cancer (IARC). were retrieved, in order that a hazard profile for each of the substances could be prepared. In many cases reliance on such sources for a complete dataset was not possible, so full literature searches were also conducted. All major biomedical, toxicological and environmental on-line databases were searched (see below). Data on the 53 pesticide metabolites have been summarised in individual hazard assessments; the individual hazard assessments form Appendices 11.1 – 11.53.

Toxicity searches

Searches to identify data on toxicity, carcinogenicity or reproductive effects were performed on the host Datastar in the major biomedical databases (see Table 12.1 for summary).

Table 12.1. Toxicity search database summary

Database	Database producer	Database label	Coverage
Medline	National Library of Medicine	MEDL	1996–present
ToxFile	Dialog Corporation AG[a]	TOXL	1900–present
Embase	Elsevier B.V.	EMED	1996–present
Biosis	Thomson Scientific	BIOL	1996–present

[a] Using data provided by the National Library of Medicine

A summary of the toxicity terms used is shown in Table 12.2. In all databases phrases and descriptors were combined using the 'and' operator with synonyms and CAS numbers for the individual pesticide or metabolite. In Medline, ToxFile and Embase, the 'with' command was used when a specific descriptor was available for each pesticide metabolite. In addition, searches were also performed using Scopus database.

Table 12.2. Summary of the standard toxicity search strategy

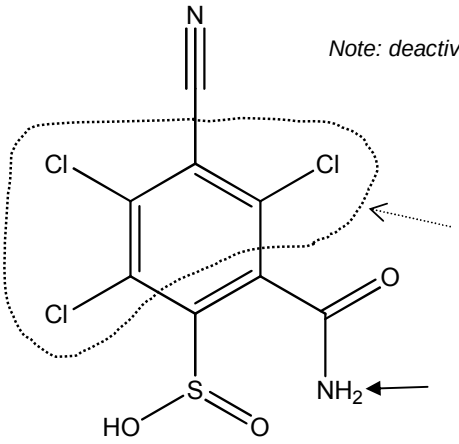
	Operator	Search Phrase/Descriptor
Medline		
Pesticides (metabolites).ti,de,ab.[a]	AND	Health adj[b] effect$1[c].ti,ab,de.
		Adverse adj effect$1.ti,ab.
CAS number.rn.		Toxicology#[d]
		Toxicity-tests#
		Toxic$8.ti,ab.
		Carcinogen$5.ti,de,ab.
		Teratogen$5.ti,de,ab.
		Mutagen$5.ti,de,ab.
		Mutagenicity-tests#
		Neurotoxic$8.ti,de,ab.
		Cytotoxic$8.ti,de,ab.
		Genotoxic$8.ti,de,ab.
		Poison$3.ti,ab.
Pesticides.de.	WITH[b]	Toxicity.de.
		Adverse adj effect$1.de.
		Poisoning.de.
Embase		
Pesticides (metabolites).ti,de,ab.	AND	Health adj effect$1.ti,de,ab.
CAS number.rn.		Adverse adj effect$1.ti,de,ab.
		Toxic$8.ti,ab.
		Toxicity#
		Toxicity-testing#
		Carcinogen$5.ti,de,ab.
		Mutagen$5.ti,de,ab.
		Teratogen$5.ti,de,ab.
		Neurotoxic$8.ti,de,ab.
		Cytotoxic$8.ti,de,ab.
		Genotoxic$8.ti,de,ab.
		Poison$3.ti,de,ab.
Pesticide.de.	WITH	Toxicity.de.
Biosis		
Pesticides (metabolites).ti,de,ab.	AND	Health adj effect$1.ti,de,ab.
CAS number.rn.		Adverse adj effect$1.ti,de,ab.
		toxic$8.ti,ab.
		Carcinogen$5.ti,de,ab.
		Teratogen$5.ti,de,ab.
		Mutagen$5.ti,de,ab.
		Neurotoxic$8.ti,de,ab.
		Cytotoxic$8.ti,de,ab.
		Genotoxic$8.ti,de,ab.
		Poison$3.ti,de,ab.
Pesticides (metabolites).de.	WITH	toxic$8.de.

[a] Letters indicate the fields searched .de. = descriptors, .ti. = title, .ab. = abstract, .rn. = registry number
[b] Proximity operators: ADJ = adjacent to; WITH = in the same sentence, words in any order; AND = in the same paragraph
[c] $1 – truncation symbol, a number indicates the number of characters allowed
[d] #- term was exploded to include all terms below in the hierarchical descriptor tree
[e] Concept code for toxicity

Appendix 14 – Prediction of Toxicity Retention or Abatement of Metabolites with Chlorine and Ozone

For each metabolite, dark arrows point to likely points of electrophilic attack. Question marks ("?") show less likely (though possible) points of attack. A dotted line on each structure shows the predicted toxic moiety. Notes by each structure indicate if an aromatic ring is likely to be deactivated, and whether a metabolite is likely to be detoxified. Toxic moieties were identified based on toxic alerts predicted by DEREK and/or the respective moiety associated with pesticidal toxicity (see S

1. R417888

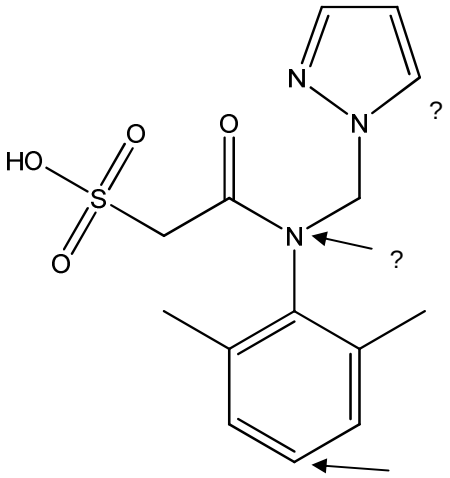


Note: deactivated ring

Not likely detoxified. [Polyhalogenated aromatic relates to carcinogenicity]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack or ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 59.7%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 52.1%)

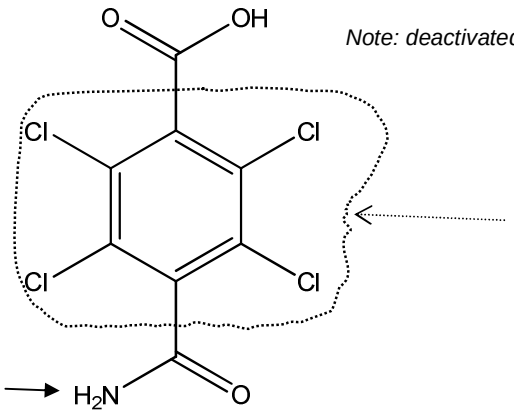
2. metazachlor sulfonic acid



Likelihood of detoxification unknown. [Developmental toxicant. Responsible moiety unknown.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 99.6%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 74.7%).

3. 3-carbamyl-1,2,4,5-tetrachlorobenzoic acid

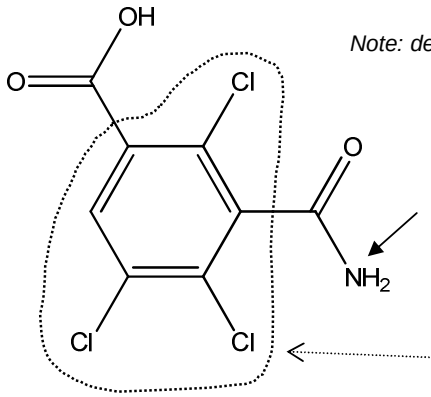


Note: deactivated ring

Not likely detoxified. [Polyhalogenated aromatic relates to carcinogenicity]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = Calc error. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = Calc error.

4. 3-carbamyl-2,4,5-trichlorobenzoic acid

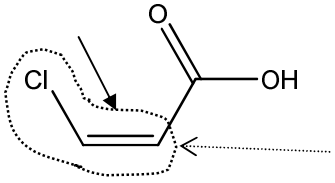


Note: deactivated ring

Not likely detoxified. [Polyhalogenated aromatic relates to carcinogenicity]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = Calc error. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = Calc error.

5. cis-3-chloroprop-2-enoic acid

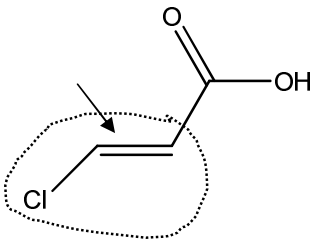


Likely detoxified. [Polyhalogenated alkene relates to carcinogenicity and mutagenicity]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams).

Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 55.7%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 30.8%).

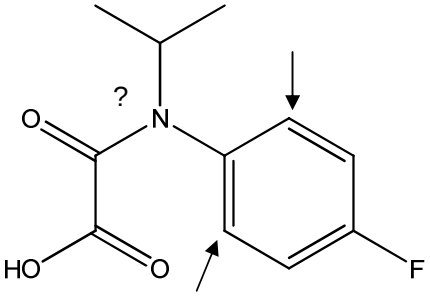
6. trans-3-chloroprop-2-enoic acid



Likely detoxified.
[Polyhalogenated alkene relates to carcinogenicity and mutagenicity]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack or ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 55.7%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 30.8%).

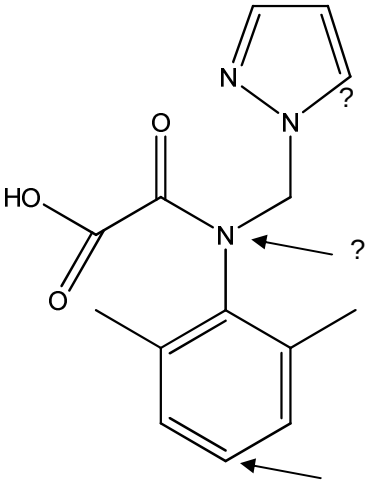
7. FOE oxalate



Likelihood of detoxification unknown.
[Corcinogen. Responsible moiety unknown.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 59.3%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 53.1%).

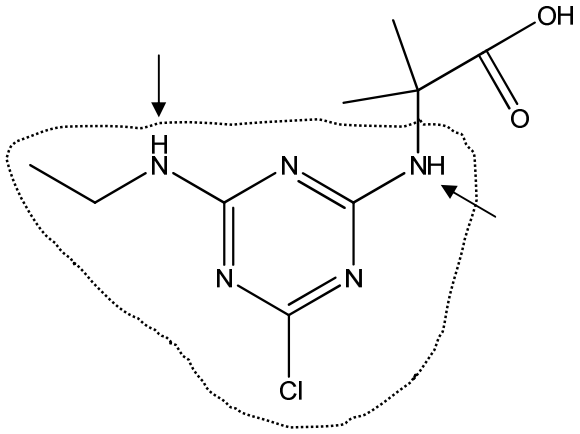
8. metazachlor oxalic acid



Likelihood of detoxification unknown.
[Developmental toxicant. Responsible moiety unknown.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 85.7%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 77.9%).

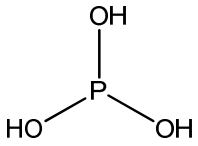
9. cyanazine acid



Likelihood of detoxification possible.
[Maintenance of alkyl side chain in triazine + chlorine. Herbicidal activity.

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 55.6%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 59.1%).

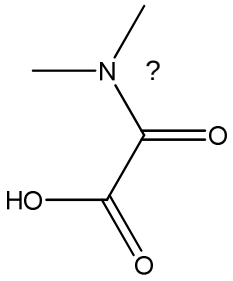
10. phosphorous acid



Likelihood of detoxification unknown.
[Whole molecule identified as active moiety/molecule.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = Calc error. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = Calc error.

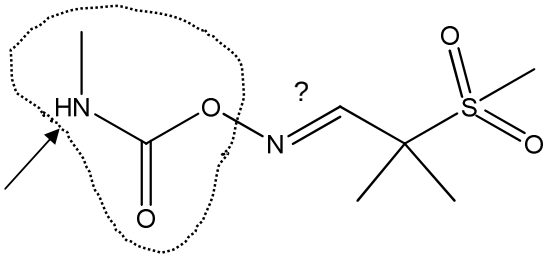
11. dimethyloxamic acid



Likelihood of detoxification unknown.
[Carcinogen plus developmental toxicant. Responsible moiety unknown.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 58.6%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 47.9%).

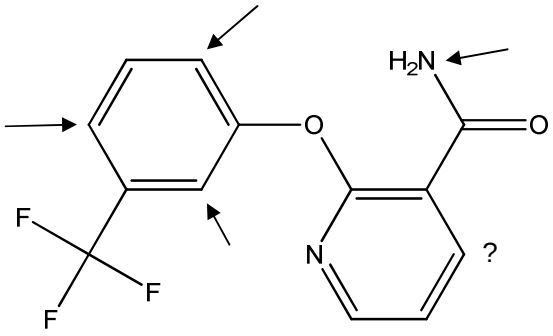
12. aldicarb sulfone



Possible detoxification.
[Pesticidal activity (insecticide). Acetylcholine esterase inhibitor (moiety shown). Developmental toxicant (moiety unknown).

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 67.3%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 39.0%).

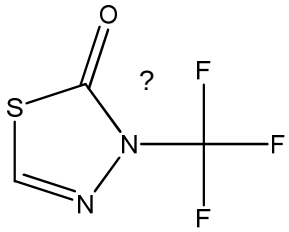
13. AE 0542291



Likelihood of detoxification <u>unknown</u>.
[Carcinogen plus developmental toxicant. Responsible moiety unknown.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 72.4%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 45.9%).

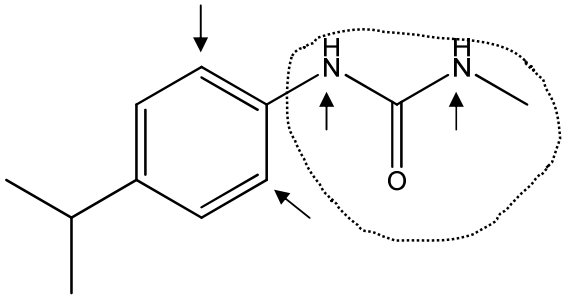
14. thiadone



Likelihood of detoxification <u>unknown</u>.
[Carcinogen plus high rat oral LD50. Responsible moiety unknown.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 50.2%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 6.5%).

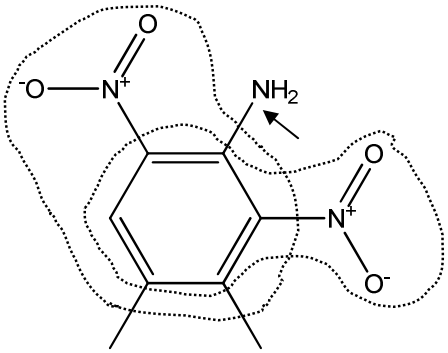
15. desmethylisoproturon



<u>Possible</u> detoxification.
[Urea herbicide moiety and herbicidal activity (moiety shown). Carcinogen (moiety unknown).

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 79.0%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 63.2%).

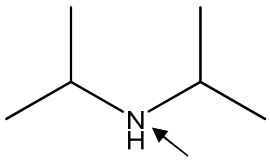
16. 2,6-dinitro-3,4-xylidine



Possible detoxification. [Aromatic nitro compound. Carcinogen and mutagen.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 95.8%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 82.5%).

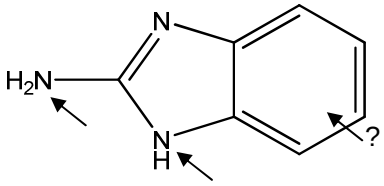
17. diisopropylamine



Likelihood of detoxification unknown. [Carcinogen plus developmental toxicant. Responsible moiety unknown.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 72.5%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 33.7%).

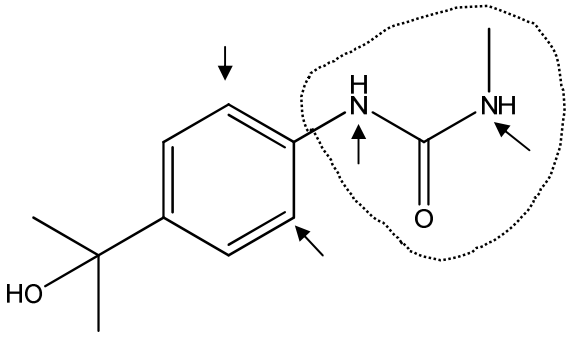
18. 2-aminobenzimidazole



Possible detoxication. [Polyaromatic amine. Carcinogen. Mutagen.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 77.5%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 70.9%)

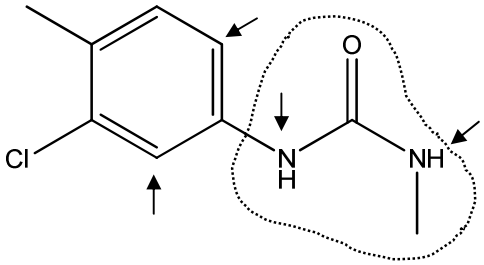
19. 3-[4-(2'-hydroxy-2'-propyl)-phenyl]-methyl urea



<u>Possible</u> detoxication.
[Urea herbicide.Herbicidal activity.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 78.8%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 65.8%).

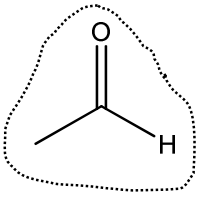
20. 3-(3-chloro-p-tolyl)-1-methylurea



<u>Possible</u> detoxication.
[Urea herbicide.Herbicidal activity.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 71.5%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 54.6%).

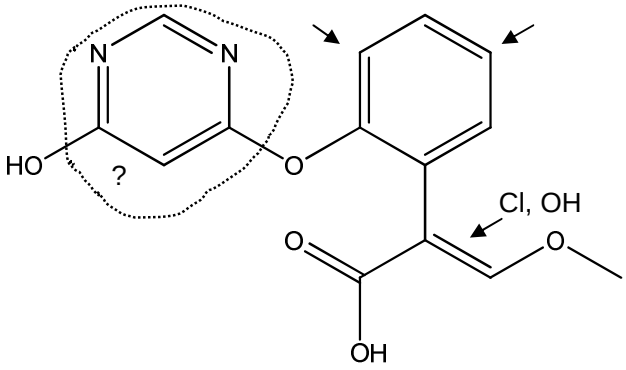
21. acetaldehyde



<u>Possible</u> detoxication.
[Alkyl aldehyde (or precursor) and mutagen. Also carcinogen (moiety unknown)]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = Calc error. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = Calc error.

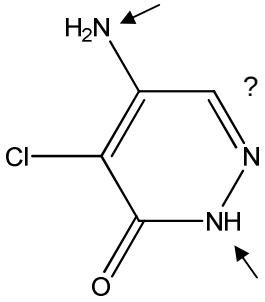
22. reference compound 10



Likelihood of detoxification <u>unknown</u>.
[Substituted pyrimidine carcinogen. Also, developmental toxicant (moiety unknown).]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 79.1%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 73.4%).

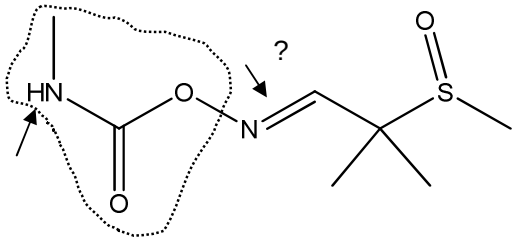
23. 5-amino-4-chloropyridazin-3(2H)-one



Likelihood of detoxification <u>unknown</u>.
[Carcinogen plus developmental toxicant. Responsible moiety unknown.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 73.4%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 60.8%).

24. aldicarb sulfoxide

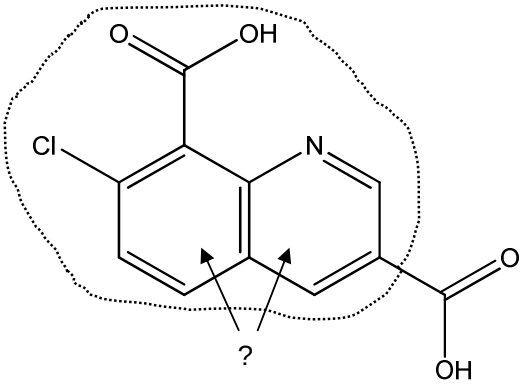


<u>Possible</u> detoxification.
[Pesticidal activity (insecticide). Acetylcholine esterase inhibitor (moiety shown). Developmental toxicant (moiety unknown).]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams).

Question mark (“?”) indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 70.5%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 46.0%).

25. BH518-2

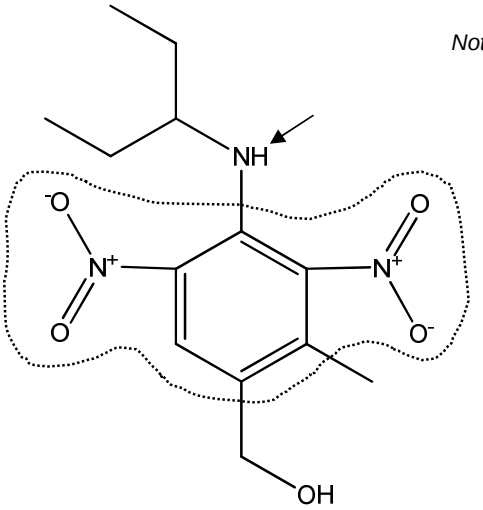


<u>Possible</u> detoxification. [Pesticidal activity (herbicide). Quinolinecarboxylic acid.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark (“?”) indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 68.9%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 63.8%).

26. 4-[(1-ethylpropyl)amino]-2-methyl-3,5-dinitro benzyl alcohol

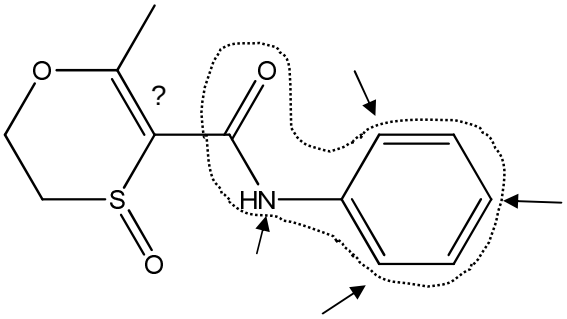
Note: deactivated ring



Likelihood of detoxification <u>unknown</u>. [Nitroaromatic. Carcinogen and mutagen.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 100%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 88.8%).

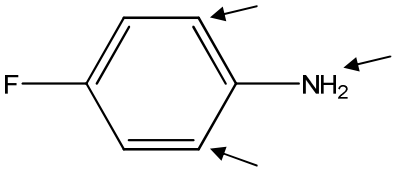
27. carboxin sulfoxide



Possible detoxification. [Pesticidal activity (fungicide).]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 90.9%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 69.0%).

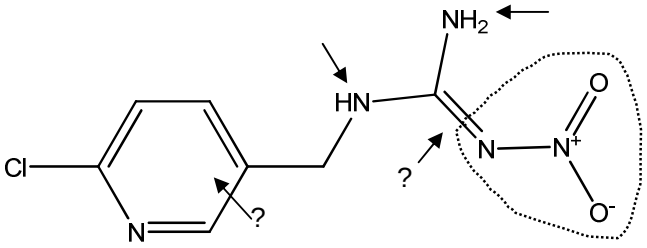
28. 4-fluoroaniline



Likelihood of detoxification unknown. [Carcinogen plus mutagen. Responsible moiety unknown.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 76.4%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 64.6%)

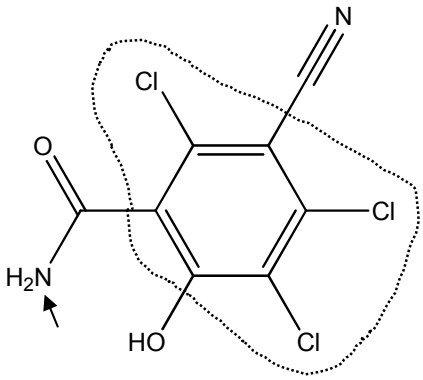
29. 1-(6-chloro-pyridine-3-ylmethyl)-N-nitro guanidine



Likelihood of detoxification unknown. [N-nitro or N-nitroso. Carcinogen plus mutagen. (Also, developmental toxicant. Responsible moiety unknown).]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 75.5%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 54.7%).

30. 3-cyano-6-hydroxy-2,4,5-trichlorobenzamide

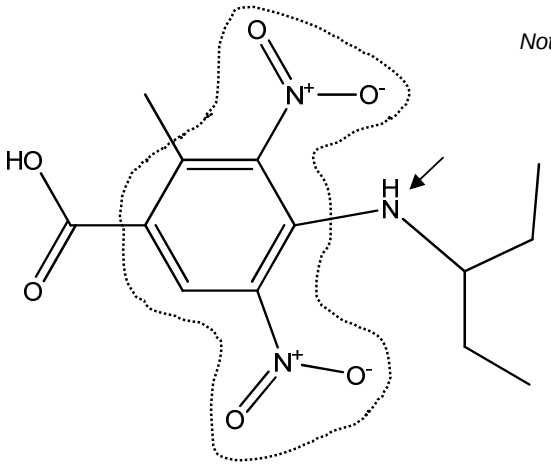


Note: deactivated ring

Likelihood of detoxification unknown.
[Polyhalogenated aromatic. Carcinogen. (Also, high rate oral LD50 (moiety unknown.)]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 60.9%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 50.7%).

31. 4-[(1-ethylpropyl)amino]-3,5-dinitro-o-toluic acid

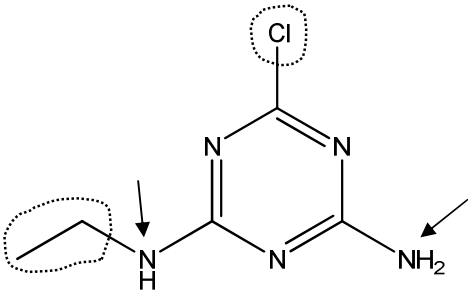


Note: deactivated ring

Likelihood of detoxification unknown.
[Aromatic nitro compound. Carcinogen and mutagen.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 95.0%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 87.4%).

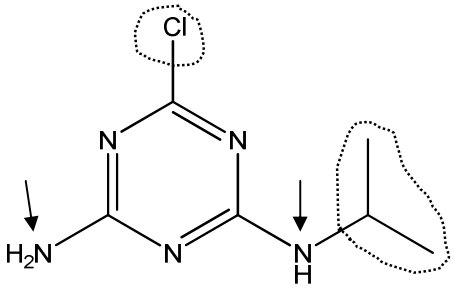
32. desisopropylatrazine



Likelihood of detoxification <u>likely with dechlorination or with dealkylation</u>.
[Maintenance of chlorine and alkyl side chain on triazine. Herbicide.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 55.9%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 47.9%).

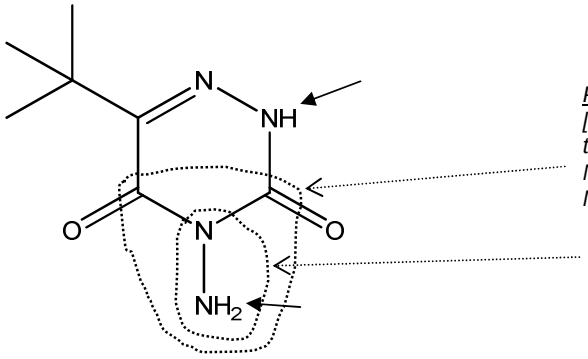
33. deethylatrazine



Likelihood of detoxification <u>likely with dechlorination or with dealkylation</u>.
[Maintenance of chlorine and alkyl side chain on triazine. Herbicide.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 55.8% Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 47.4%).

34. diketo metribuzin

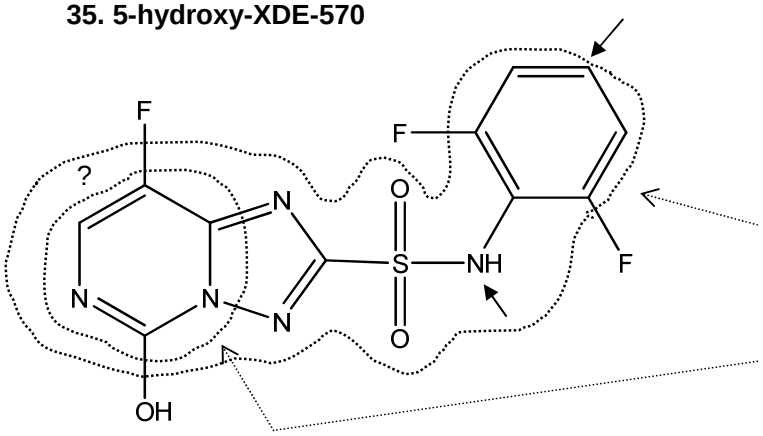


<u>Possible</u> detoxification.
[Hydrazine. Carcinogen and teratogen.
N-amino heterocyclic. Mutagen.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 63.0%.

Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 53.9%).

35. 5-hydroxy-XDE-570

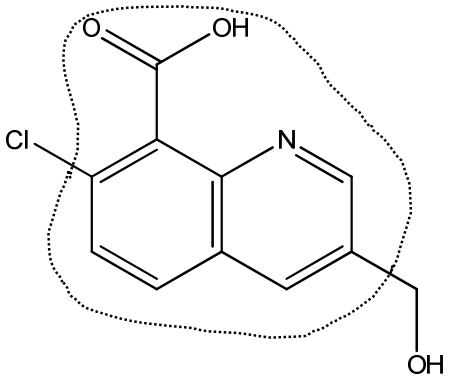


Likelihood of detoxification unknown.
[Substituted pyrimidine (or purine). Carcinogen.

Herbicidal activity.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 82.3%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 66.4%).

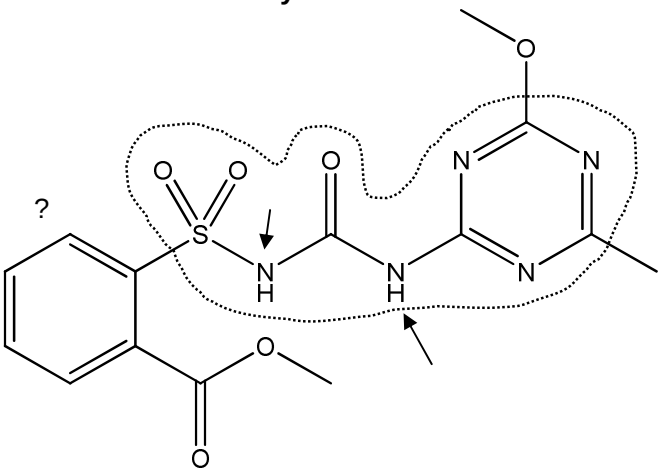
36. BH518-4



Likelihood of detoxification unknown.
[Pesticidal activity (herbicide).]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 79.3%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 66.6%).

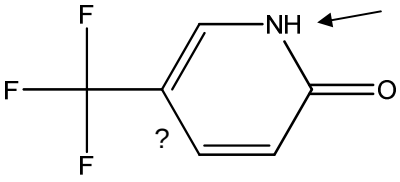
37. metsulfuron-methyl



<u>Possible</u> detoxification. [Pesticidal activity (herbicide). Sulfonylurea moeity.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 82.5%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 66.3%).

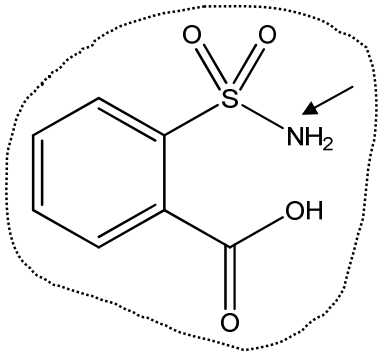
38. 5-trifluoromethyl-pyrid-2-one



Likelihood of detoxification <u>unknown</u>. [Mutagen, carcinogen, high rat oral LD50, developmental toxicant. Moieties unknown.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 64.6%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 34.7%).

39. IN-D5119



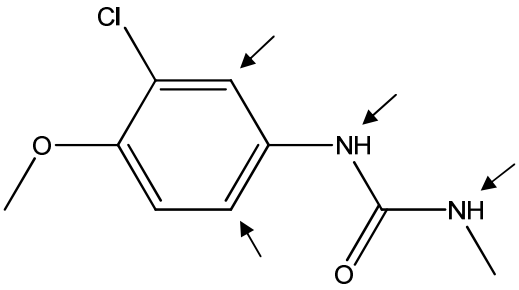
Note: Deactivated ring

Likelihood of detoxification <u>unknown</u>. [Teratogen.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams).

Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 75.0%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 45.0%).

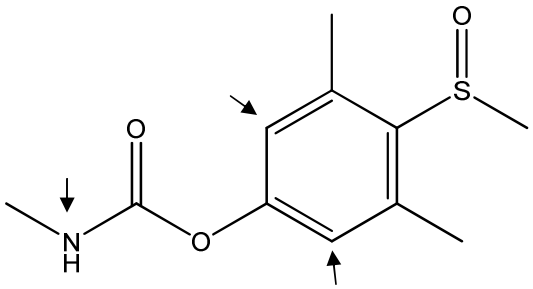
40. demethyl metoxuron



Likelihood of detoxification unknown.
[High rat oral LD50 and carcinogen. (Moiety unknown.)]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 70.0%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 56.0%).

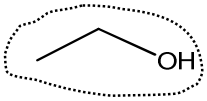
41. methiocarb sulfoxide



Likelihood of detoxification unknown.
[High rat oral LD50 and carcinogen. (Moiety unknown.)]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack y ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 84.5%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 58.2%)

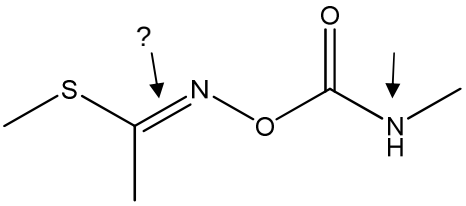
42. ethanol



Likelihood of detoxification unknown.
[Teratogen.]

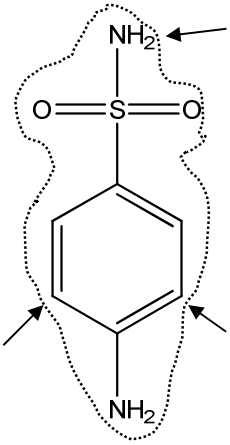
(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 68.2%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 55.2%)

43. methomyl



(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 58.2%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 45.5%)

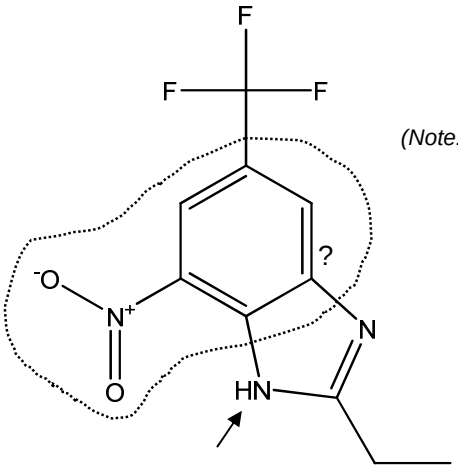
44. sulphanilamide



Likelihood of detoxification unknown.
[Thyroid toxicity.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 79.8%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 69.5%)

45. 2-ethyl-7-nitro-5-(trifluoromethyl) benzimidazole

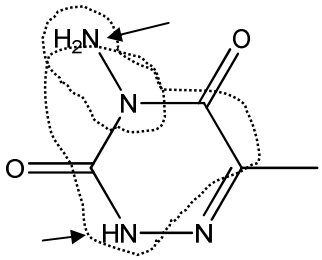


(Note: deactivated ring)

Likelihood of detoxification unknown.
[Nitro aromatic.Carcinogen. Mutagen. Also, high rat oral LD50 (moiety unknown).]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 68.4%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 46.0%)

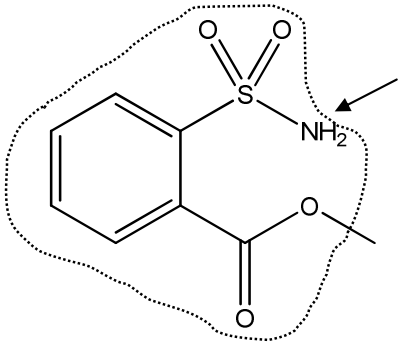
46. CGA 294849



Likelihood of detoxification possible.
[Hydrazine. Carcinogen. Teratogen. N-amino heterocyclic. Mutagen.)

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 68.5%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 57.5%)

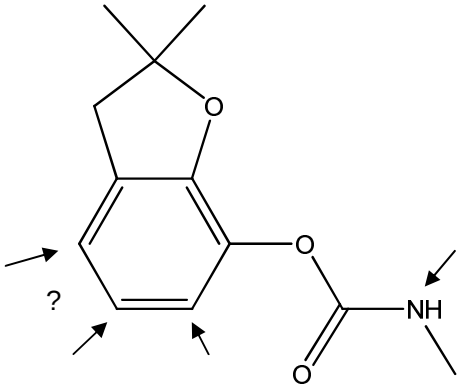
47. IN-D5803



Likelihood of detoxification possible.
[Aryl sulphonabmide (most of molecule.) Teratogen.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 70.7%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 41.1%)

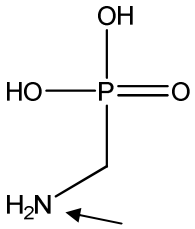
48. carbofuran



Likelihood of detoxification unknown.
[High rat oral LD50 and carcinogen. (Moiety unknown.)]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Question mark ("?") indicates possible point of attack. Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 79.0%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 55.9%)

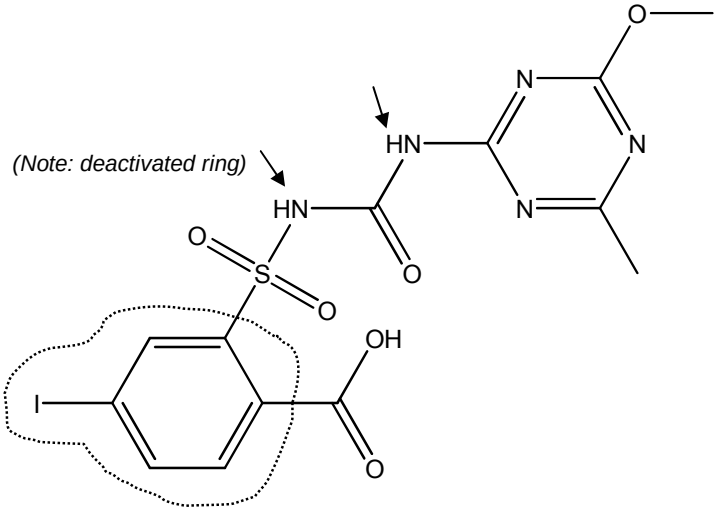
49. aminomethylphosphonic acid



Likelihood of detoxification unknown.
[High rat oral LD50 and carcinogen. (Moiety unknown.)]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 77.4%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 55.1%)

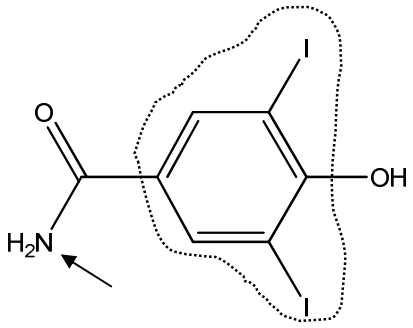
50. AE F145740



Likelihood of detoxification <u>not likely</u>.
[Aromatic iodo compound. Thyroid toxicity.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 75.0%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 68.1%)

51. 3,5-diiodo-4-hydroxybenzamide

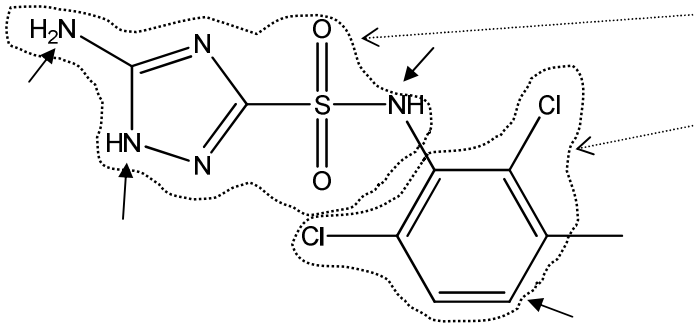


(Note: deactivated ring)

Likelihood of detoxification <u>not likely</u>.
[Poly halogenated aromatic. Carcinogen. Aromatic iodo compound. Thyroid toxicity.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 63.7%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 53.3%)

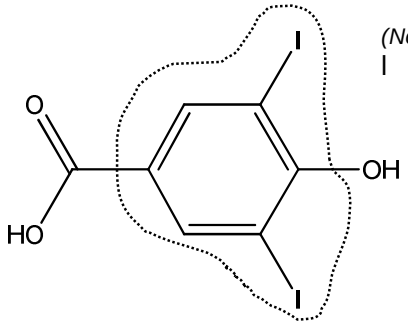
52. ATSA



Likelihood of detoxification <u>possible</u>.
[Aminoaryl sulphonamide (or precursor). Thyroid toxicity.
Poly halogenated aromatic. Carcinogen.

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 72.6%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 73.6%)

53. 3,5-diiodo-4-hydroxybenzoic acid



(Note: deactivated ring)

Likelihood of detoxification <u>unknown</u>.
[Iodo aromatic. Thyroid toxicity.]

(Dashed line is likely moiety causing toxicity (ref. C. Sinclair). Solid arrow shows possible primary point(s) of attack by ozone and/or chlorine (ref. C. Adams). Predicted removal via ozonation (typical conditions) (per QSPR calculation) = 58.5%. Predicted removal via chlorination (typical conditions) (per QSPR calculation) = 51.8%)