GENDER SENSITIVITY OF XENOBIOTICS

Summary of the Literature

In order to conserve animals in acute toxicity testing, OECD experts have recommended the use test animals of a single sex. Sex as a cause of differences in metabolism, transformation, and toxicity, have been reviewed by a number of authors. These authors have compiled available data on gender sensitivity to toxicants in rats, mice and humans. See, for example, Reviews by Salem, Trimbell, Sipes and Gandolpho, DeBethizy and Hayes, and Moser (1, 2, 3, 4, 5). However, we are not aware of systematic investigations into differences in sensitivity for lethality of xenobiotics of males and females across chemicals.

Surveys of the literature show that generally, the responses in male and female rats are similar. When differences in sensitivity occur, it is often the female that is more sensitive (Kedderis and Mugford, 6) Summarizing acute toxicity data on 766 chemicals, no significant sexual differences are noted in 711 cases, constituting 93% of the cases. When differences are noted, females are more sensitive in 42 cases, while males are more sensitive in 13 cases. (See Table 1.) In other tabulations, for 91 chemicals the female average LD50 value is slightly lower than that for males, while for 143 chemicals, the opposite is true. In some cases, dissimilarities in sensitivity between male and female rats can be significant. For example, in a comparison of male and female rat oral and dermal LD50 values for pesticides (EPA , 7), 14 out of 79 pesticides showed significant differences in sensitivity in male and female rats. In this report, difference in response was deemed to be significant if there was no overlap of the 95% confidence intervals characterizing each sex's response. As shown in Tables 1 and 2, for 11 cases, females were more sensitive and for 3 cases, males were more sensitive. Properties and structures for the chemicals in Table 2 are given in Table 2A.. The three chemicals which showed greater sensitivity in the male rat were Landrin, a carbamate insecticide, Triflumizole, an imidazole fungicide, and vitamin D3, a steroidal pesticide. Additional disparities in sex sensitivity were seen for many of the rest of the chemicals in the pesticide data base, although for these chemicals, 95% confidence intervals overlapped to some extent. While these data suggest that the sexes are not equally sensitive to all of the chemicals tested, no clear cut generalizations about sex sensitivity could be made; although females were often more sensitive, this was not always true.

The published literature records cases when male rodents are more sensitive to xenobiotics than females. A detailed review of the metabolism of Chlorpyrifos can be found in Moser. Timbrell notes that Chlorpyrifos is more acutely toxic to male rats than to females. Differences in the way that vital organs react to toxins can also have a significant impact on overall toxicity. Chloroform induces nephrotoxicity in male mice, but not females; chloroform is converted to a reactive intermediate (phosgene) an order of magnitude faster by microsomes from male mouse kidneys than in those from female mice (Sipes and Gandolpho). Metabolic differences due to gender can also have an effect on sensitivity for acute effects. The insecticides aldrin and heptachlor are metabolized more rapidly to the toxic epoxide forms in male rats. These chemicals demonstrate a lower toxicity in the female rat (Trimbell).

Sensitivity Differences in Avian Species:

In a separate review, Elwood Hill (8) compared the toxicity of ten insecticides in birds (sex unspecified). The list contained both organophosphate and carbamate pesticides.
(Tables 3 and 3A). The redwing blackbird has lower specific hepatic microsomal monooxygenase activity than most other animals (for example, rock dove, chukar, mallard, or ring-necked pheasant). By analogy to female rats with their lower biotransformation capacity, one would expect the redwing blackbird to have lower LD50 values for these insecticides than the other species. In fact, the redwing blackbird was more sensitive than the other avian species to seven chemicals. However, for two chemicals, chlorpyrifos and mexacarbate, the redwing blackbird was generally less sensitive than the other species.

Biotransformation and Differences in Sensitivity:

If gender differences are seen in toxic responses to xenobiotics, differences in biotransformation are the probable cause. Because male rats metabolize most foreign compounds faster than females, one would expect the biological half-life of most xenobiotics to be longer in the female than the male rat. However, if a metabolite or intermediate is responsible for the toxic response, male rats would be expected to show the greater susceptibility (Sipes and Gandolfo).

In general, CYP mediated reactions lead to detoxification and subsequent excretion of xenobiotics (phase I metabolism). For example, certain organophosphate pesticides are detoxified by glutathione S-transferases. However, CYP mediated metabolism can also cause formation of reactive metabolites. Female rats are known to have 10 - 30% less total CYP as compared with male rats. (Kedderis and Mugford).

Phase II conjugative enzymes, i.e. sulfotransferases, glutathione S-transferases, and glucuronyltransferases, also play a role in detoxification. Sex-dependent differences have also been found in expression of phase II enzymes. When such sex-dependent differences are seen, it is generally the male rats which have higher enzyme activities. For example, glutathione protects tissues against electrophilic attack by xenobiotics. DeBethizy and Hayes note that glutathione conjugating activity toward dichloronitrobenzene is two- to three-fold higher in male than female rats.

Biotransformation does not always lead to detoxification. Examples of activation of xenobiotics to their toxic forms by mixed function oxidase enzymes are:

- epoxidation of chlorobenzene and coumarin to generate hepatotoxic metabolites,

- oxidative group transfer of certain organophosphorous pesticides to the toxic organophosphate, e.g. conversion of parathion to paraoxon,

- reductive dechlorination of carbon tetrachloride to a trichloro methyl free radical,

- oxidative dechlorination of chloroform to phosgene,

- activation of ethyl carbamate to (urethan)

However, many of these same chemicals are also detoxified by cytochrome P450 by conversion to less toxic metabolites. In some cases, the same enzyme may catalyze activation and detoxification reactions for a given chemical. The resulting toxic effect of a xenobiotic chemical is thus due to a balance between metabolic activation and deactivation (Casarett and Doull, 9).

Although female rats generally have less total CYP activity than males, there are important exceptions. For example, microsomal 16-hydroxylase is male specific and is not expressed in females. Whereas steriod sulfate 15 hydroxylase occurs in higher concentrations in females. One could speculate that these differences may account for the fact that vitamin D3 is more toxic in males than females.

De Bethizy and Hayes also note that phase II conjugation of xenobiotics maky not always lead to more rapid excretion of the conjugated metabolite. In fact, some compounds are toxic only after conjugation with glutathione. Glutathional conjugates which are implicated in nephrotoxicity would be likely to ;show greater toxicity in males than females.

Choice of Sex for Acute Toxicity Testing:

As noted above, fourteen pesticides, from a sample of 84, were found to exhibit significant differences in sensitivity between male and female rats (Table 2). When they occur, dissimilarities in sensitivity of male and female rats can also have important implications for regulation. In five of the fourteen cases, the disparity of response was such that had only one sex been tested, and it was the least sensitive sex, the chemical would have been assigned for classification to a less toxic class.

The revised test guideline #425 uses a single sex, usually females. If the investigator has a priori reasons to believe that males may be more sensitive than the other, then it may be used for testing. Female rats have a lower relative detoxification capacity for most chemicals, as measured by specific activity of their mixed function oxidase enzymes. Therefore, for chemicals which are direct acting in their toxic mechanism, females would generally be the most sensitive. However, if metabolic activation is required for a chemical's toxicity, consideration must be given as to whether the preferred sex for testing is the male.

Table 1. LD50 sensitivity of the sexes

(See Lipnick, R.L., et al. 1995 Comparison of the up-and-down, conventional LD50, and fixed-dose acute toxicity procedures. Fd. Chem. Toxicol. 33: 223-231).

Author	**No. Chemicals**	**LD50 Average (mg/kg)**		
		Females	**Males**	
DePass et al., 1984	91	2130	2470	
Weil et al., 1953	143	8960	8360	
Weighted Average	234	6313	6069	
		LD50 Sensitivity of the Sexes		
		Sexes Same	**Sex More Sensitive**	
			Female	**Male**
Bruce, 1985	48	35	13	0
EPA, 1991	79	65	11	3
HSE, 1999	449	446	1	2
Lipnick et al., 1995	20	18	0	2
Muller & Kley, 1982	170	147	17	6
Totals	766	711 (93%)	42	13

Table 2. **Chemicals without overlapping male and female LD50 (95% confidence limits)**

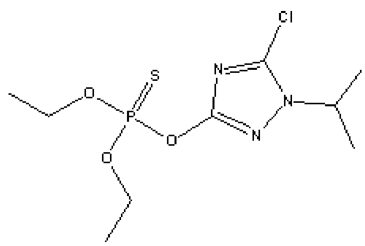
CHEMICAL NAME	CHEMICAL CLASS	USE	MALE LD50 mg/kg	FEMALE mg/kg
1. Isazofos technical (93+%)	Organophosphate	Insecticide	118.68	48.21
2. Trimethacarb	Carbamate	Insecticide	7.20	9.30
3. Flusilazole (97%)	Fluorophenyl triazole silane	Fungicide	1110.00	674.00
4. Cadusafos (94.9%) (in corn oil)	Organophosphate	Insecticide	47.50	20.10
5. Cycloate technical (98%)	Carbamate	Herbicide	3200.00	2275.00
6. Clomazone (88.8% a.i.)	Chlorophenyl isoxazolidinone	Herbicide	2077.00	1369.00
7. Troysan polyphase (99%)	Iodo-acetylenic carbamate	Fungicide/wood preservative	1795.00	1065.00
8. Parathion technical (in corn oil)	Organophosphate	Insecticide	10.80	2.52
9. Chlorethoxyfos (86% a.i.)	Organophosphate	Insecticide	4.60	1.80
10. ASPON technical (90%); (inerts 10%)	Organophosphate	Insecticide	2800.00	740.00
11. Triflumizol technical	Imidazole	Fungicide	1057.00	1780.00

Table 2. Chemicals without overlapping male and female LD50 (95% Confidence limits) (cont'd.)

CHEMICAL NAME	CHEMICAL CLASS	USE	MALE LD50 mg/kg	FEMALE mg/kg
12 Thiodicarb (in methyl cellulose)	Carbamate	Insecticide	129.00	59.10
13. Vitamin D3 technical	Steroid	Antirachitic	352.00	619.00

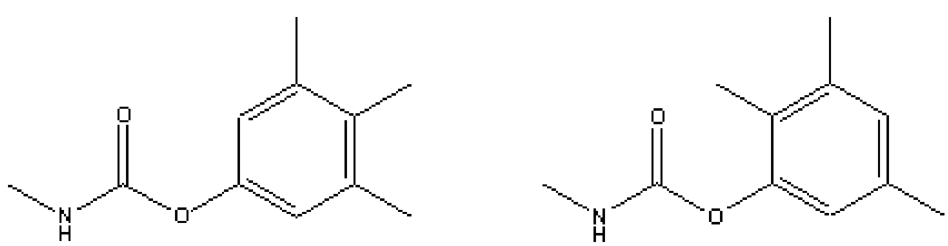
Table 2A. Identification of Chemicals in Table 2

1) CGA-123 technical
This substance is identified in the MRID as CGA 12223 from Ciba, Ltd.
According to the Farm Chemicals Handbook (FCH), vol.86 (2000), the following information was obtained :
Common Name: Isazofos
Chemical Name: O -5-chloro- 1-isopropyl-1H-1,2,4-triazol-3-yl-O,O-diethyl-phosphorothioate
CAS No. 42509-80-8
Chemical Class: organophosphate
Use: Insecticide
Structure:



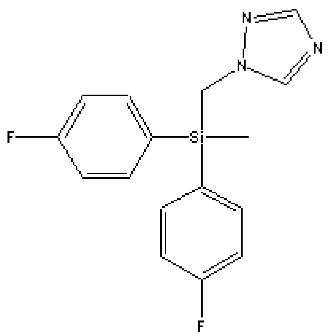
Empirical Formula: C9 H17 N3 P O3 S Cl
Molecular Weight: 313.5

2) El-919
Tradename (of Shell): Landrin
Common Name: Trimethacarb
Chemical Name: 3,4,5- trimethylphenyl methylcarbamate
CAS No. 2655-15-4
Chemical Class: carbamate
Use: Insecticide
Structure:



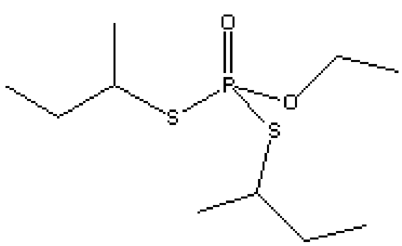
(*Note: The pesticide is a mixture of both forms, 3,4,5- and 2,3,5- trimethylphenyl methylcarbamate)*
Empirical Formula: C11 H15 O2 N
Molecular Weight: 182

3) 1-[[bis (4-fluorophenyl) methylsilyl] methyl]-1H-1,2,4-triazole
CAS No. 85509-19-9
Common Name: Flusilazole
Tradename: Nustar
Chemical Class: fluorophenyl triazole silane
Use: Fungicide
Structure:



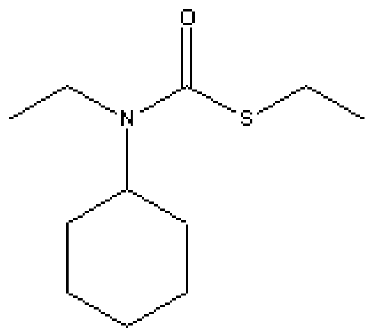
Empirical Formula: C16 H15 F2 N3 Si
Molecular Weight: 315.4

4) FMC 67825
Tradename: Rugby ; Apache
Common Name: Cadusafos
Chemical Name: O- ethyl-S,S- di-sec-butyl phosphorodithioate
Chemical Class: organophosphate
Use: Insecticide
Structure:



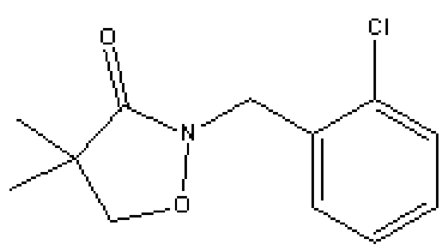
Empirical Formula: C10 H23 P O2 S2
Molecular Weight: 270

5) Cycloate technical
Chemical Name: S-ethyl cyclohexyl (ethyl) thiocarbamate
CAS No. 1134-23-2
Chemical Class: carbamate
Use: Herbicide
Structure:



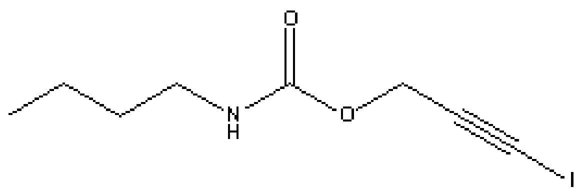
Empirical Formula: C11 H21 N O S
Molecular Weight: 204

6) FMC 57020
Tradename: Command
Common Name: Clomazone
Chemical Name: 2- [(2-chlorophenyl) methyl]-4,4-dimethyl -3-isoxazolidinone
Chemical Class: chlorophenyl isoxazolidinone
CAS No. 81777-89-1
Use: Herbicide
Structure:



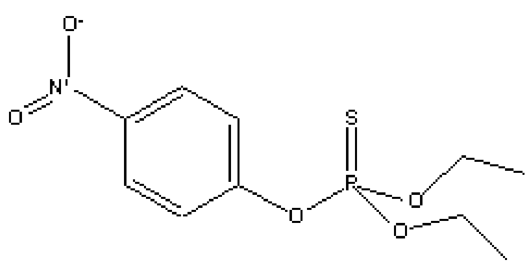
Empirical Formula: C12 H14 N O2 Cl
Molecular Weight: 239.5

7) 3-iodo-2-propynyl butylcarbamate
Complete Chemical Name: 3-iodo-2-propynyl N-n-butyl carbamate
Tradename: Troysan polyphase
Chemical Class: iodo-acetylenic carbamate
Use: fungicide/ wood preservative
Structure:



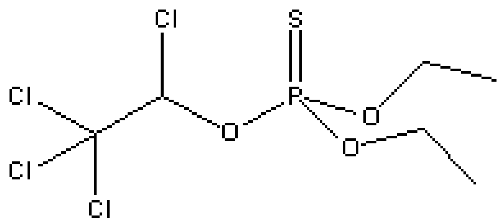
Empirical Formula: C8 H12 O2 N I
Molecular Weight: 281

8) Parathion technical
Chemical Name: O, O-diethyl- O-(4-nitrophenyl) phosphorothioate
CAS No. 56-38-2
Tradename: Thiophos
Chemical Class: organophosphate
Use: Insecticide
Structure:



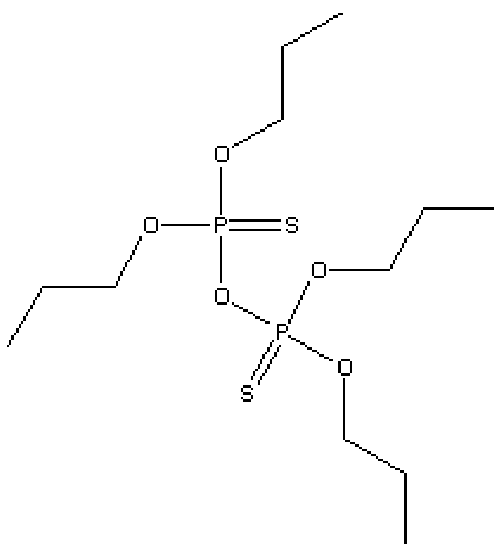
Empirical Formula: C10 H14 N PO5 S
Molecular Weight: 291

9) Fortress (tradename- Dupont)
Common Name: Chlorethoxyfos
Chemical Name: O,O-diethyl-O-(1,2,2,2-tetrachloroethyl) phosphorothioate
Chemical Class: organophosphate
Use: Insecticide
Structure:



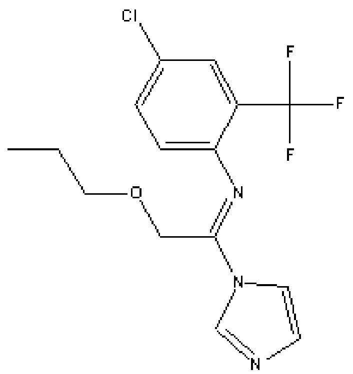
Empirical Formula: C6 H11 P O3 S Cl4
Molecular Weight: 336

10) O,O,O,O-tetrapropyl dithiopyrophosphate
CAS No. 3244-90-4
Tradename: ASPON technical (Stauffer Chemical Co.)-- discontinued 1987 by Stauffer.
Chemical Class: Organophosphate
Use: Insecticide
Structure:



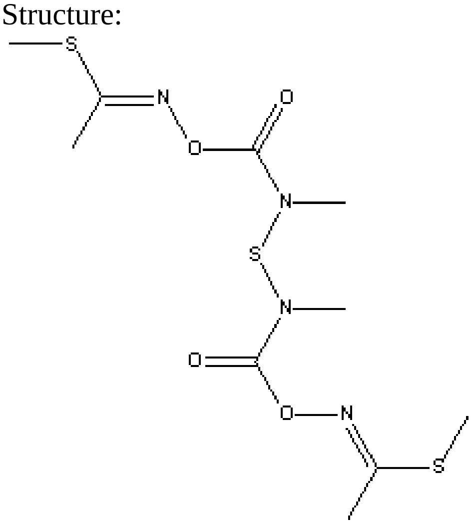
Empirical Formula: C12 H28 O5 P2 S2
Molecular Weight: 378

11) Triflumizole
Chemical Name: (E)- 4-chloro-aaa- trifluoro-N-(1-imidazole)-1 yl- 2-propoxy-ethylidene-o-toluidine
CAS No. 99387-89-0
Chemical Class: Imidazole
Use: Fungicide
Structure:



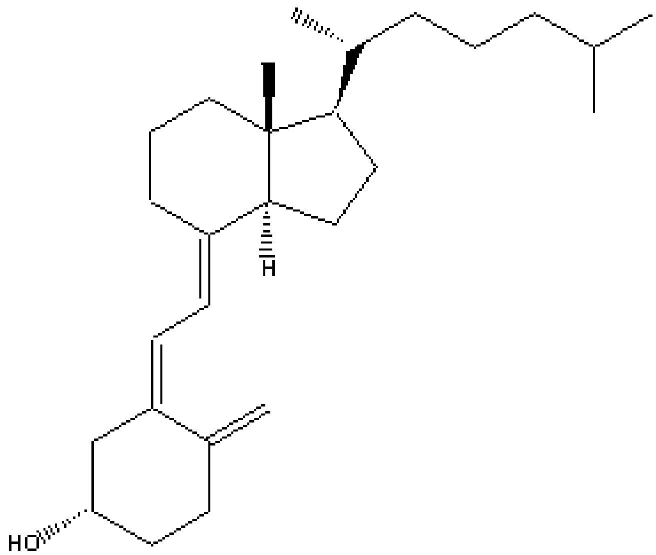
Empirical Formula: C15 H15 N3 O Cl F3
Molecular Weight: 345.5

12) Larvin (tradename / Rhone-Poulenc)
Common Name: Thiodicarb
Chemical Name: dimethyl N,N-(thiobis (methylimino) carbonyloxy) bis-ethanimidothioate)
CAS No. 59669-26-0
Chemical Class: Carbamate
Use: Insecticide
Structure:



Empirical Formula: C10 H18 N4 S3 O4
Molecular Weight: 354

13) Vitamin D3
Chemical Names: (3b,5Z,7E)-9,10-secocholesta-5,7,10-(19)-trien-3-ol;
or activated 7-dehydro-cholesterol; or cholcalciferol
Use (Merck Index, p.1711): antirachitic
Structure:



Empirical Formula: C27 H44 O
Molecular Weight: 385

* References:

1. Farm Chemicals Handbook, vol.86 (2000)
2. Merck Index, 12th edition (1996)

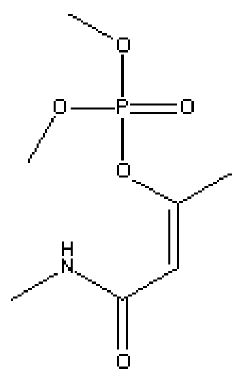
Table 3. Most sensitive cases.

Pesticide	Red-winged blackbird	Other avian species
Monocrotophos	**X**	
Dicrotophos	**X**	
Parathion		**Mallard**
EPN		**Ring-necked pheasant**
Propoxur	**X**	
Chlorpyrifos		**European starling**
Fenthion	**X**	
Temephos	**X**	**Ring-necked pheasant***
Landrin	**X**	
Mexacarbate		**Ring-necked pheasant, Chukar, Rock dove**

* Red-winged black bird and Ring-necked pheasant are very close in sensitivity.

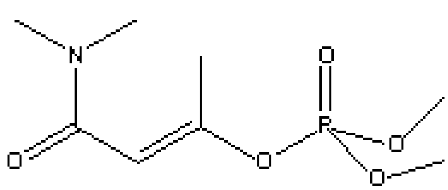
Table 3A. Identification of Chemicals in Table 3 *

1) Monocrotophos (common name)
Chemical Name: dimethyl (E)-1-methyl-2-(methylcarbamoyl) vinylphosphate
CAS No. 6923-22-4
Chemical Class: Organophosphate
Use: Insecticide
Structure:



Empirical Formula: C7 H14 P O5 N
Molecular Weight: 223

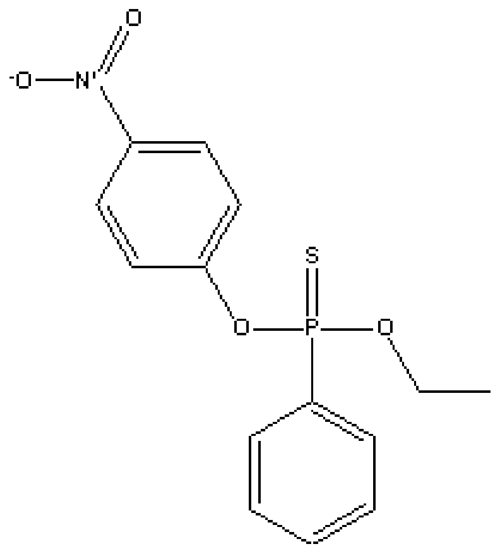
2) Dicrotophos (common name)
Chemical Name: (E)-2-dimethylcarbamoyl - 1- methylvinyl dimethylphosphate
CAS No. 141-66-2
Chemical Class: Organophosphate
Use: Insecticide
Structure:



Empirical Formula: C8 H16 P O5 N
Molecular Weight: 237

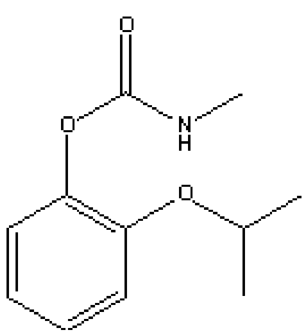
3) Parathion ------(same as 8 in Table 2A)

4) EPN (common name)
Chemical Name: O-ethyl-O- 4-nitrophenyl phenylphosphonothioate
CAS No. 2104-64-5
Chemical Class: Organophosphate
Use: Insecticide
Structure:



Empirical Formula: C14 H14 N O4 P S
Molecular Weight: 323

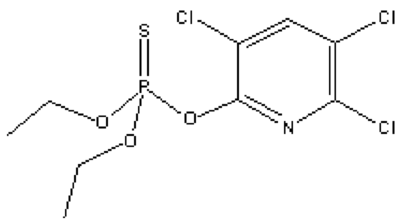
5) Propoxur (common name)
Chemical Name: 2-(1- methylethoxy) phenyl nethylcarbamate
CAS No. 114-26-1
Chemical Class: Carbamate
Use: Insecticide
Structure:



Empirical Formula: C11 H15 N O3
Molecular Weight: 209

6) Chlorpyrifos (common name)

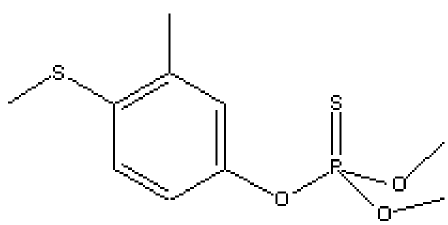
Chemical Name: O,O-diethyl- O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate
CAS No. 2921-88-2
Chemical Class: Organophosphate
Use: Insecticide
Structure:



Empirical Formula: C9 H11 Cl3 N P O3 S
Molecular Weight: 350.6

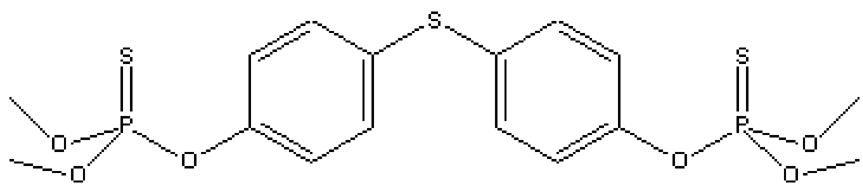
7) Fenthion (common name)

Chemical Name: O,O- dimethyl-O- [3-methyl-4-(methylthio) phenyl] phosphorothioate
CAS No. 55-38-9
Chemical Class: Organophosphate
Use: Insecticide
Structure:



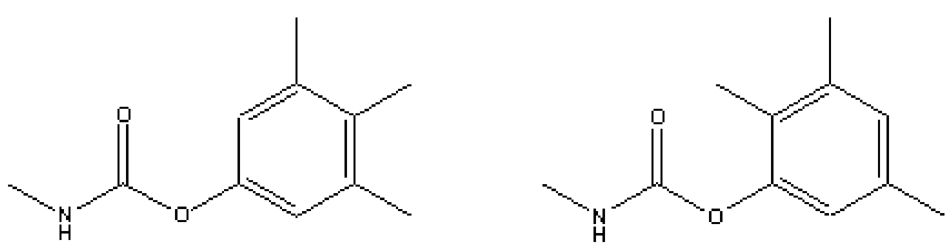
Empirical Formula: C10 H15 P O3 S2
Molecular Weight: 278

8) Temephos (common name)
Chemical Name: O,O- thiodo-4,1-phenylene- O,O,O',O'-tetramethyl-phosphorothioate
CAS No. 3383-96-8
Chemical Class: Organophosphate
Use: Insecticide
Structure:



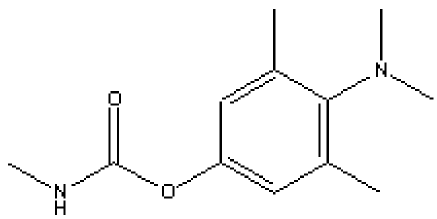
Empirical Formula: C16 H20 P2 S3 O6
Molecular Weight: 466

9) Landrin (tradename of Shell) - discontinued by Shell
Common Name: trimethacarb
Chemical Name: 3,4,5- trimethylphenyl methyl carbamate
CAS No. 2655- 15- 4
Chemical Class: Carbamate
Use: Insecticide
Structure:



(Note: The pesticide is a mixture of both forms, 3,4,5- and 2,3,5- trimethylphenyl methylcarbamate)
Empirical Formula: C11 H15 O2 N
Molecular Weight: 193

10) Mexacarbate ; Zectram
Chemical Name: 4- dimethylamino-3,5-xylyl methylcarbamate
Chemical Class: Carbamate
Use: Insecticide
Structure:



Empirical Formula: C12 H18 N2 O2
Molecular Weight: 222.3

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