RESPONSE TO COMMENTS ON INTERACTION PROFILE FOR ATRAZINE, DEETHYLATRAZINE, DIAZINON, NITRATE, AND SIMAZINE SUBMITTED BY THE ENVIRONMENTAL PROTECTION AGENCY (EPA)/OFFICE OF PESTICIDES PROGRAMS (OPP)

(The EPA comments were originally addressing the "Interaction Profile for: Atrazine, Deethylatrazine, Diazinon, Nitrate, and Simazine" and "Interaction Profile for Chlorpyrifos, Lead, Mercury, and Methylmercury" in one memorandum. For clarity, the comments pertaining to each profile were separated and responses addressed accordingly.)

Draft Interaction Profile for Atrazine, Deethylatrazine, Diazinon, Nitrate, and Simazine

Comments 1

We have reviewed the following two Draft Interaction Profiles (DIPs): "Interaction Profile for: Atrazine, Deethylatrazine, Diazinon, Nitrate, and Simazine" and "Interaction Profile for Chlorpyrifos, Lead, Mercury, and Methylmercury". Overall, we believe that the DIPs issued by ATSDR provide a valuable summary of some of the available literature concerning potential interactions between these substances, particularly with respect to ecotoxicological effects and a variety of *in vitro* studies. We particularly agree with many of the ATSDR's conclusions regarding the joint toxicity and additivity of atrazine, its deethylatrazine metabolite, and simazine. As stated in the ATSDR document, the EPA's Office of Pesticide Programs has concluded that the these triazines act by a common mechanism of action, suppressing the luteinizing hormone ovulatory surge and have an effect on reproductive function and reproductive development. We agree with ATSDR's conclusion in this regard and are actively developing a cumulative risk assessment for the triazine herbicides which accounts for this joint toxicity on this basis.

Response 1

No response needed.

Comment 2

We first note that FQPA requires that, in order for a group of chemicals to be considered a Common Mechanism Group (CMG), a common mechanism of action – not simply a potentiation – must exist. For pesticides and EPA's Office of Pesticide Programs, this means that the chemicals *within the group must cause a common toxic effect(s) by the same, or essentially the same, sequence of major biochemical events* (i.e., interpreted as mode of action). This definition is <u>different</u> from the way in which most of the rest of EPA -- in other contexts -- has defined cumulative risk assessment. Thus, we believe that it is important that the document indicate this clearly and directly by removing the reference to FQPA from the preface. Specifically, we would like the document to

(i) remove references to the FQPA from the first paragraph in the preface; and

(ii) change the first sentence of the second paragraph in the preface from:

To carry out these legislative mandates, ATSDR's Division of Toxicology …

to

To carry out the legislative mandate under CERCLA, ATSDR's Division of Toxicology …

Response 2
Following the last set of public comments on interaction profiles, **ATSDR no longer cites the Food Quality Protection Act (FQPA)** as a mandate for its mixtures activities. ATSDR mention of FQPA was in support of identifying mixtures issues as being important. Since ATSDR does not work directly under the FQPA authority, the reference was removed.

Comment 3
Secondly, we note and have concerns that much of the evidence for an interaction provided in the DIP is derived from *in vitro* studies, non-mammalian *in vivo* studies, or in studies at concentrations that are not relevant to or associated with actual drinking water concentrations.

We offer the following specific comments with respect to these latter concerns:
Regarding the potentiating effects of atrazine on the toxicity of diazinon.

None of the cited studies in support of putative joint interactions are based on mammalian studies. Instead, study citations using midge (*Chirononus tentans*) larvae in 96 hour static toxicity tests assessed acute neurotoxicity based on the inability of midges to perform normal swimming motions. These studies were done at concentrations that far exceed those relevant for actual drinking water sources: atrazine was tested in the 40-200 ppb (far higher than the 3 ppb Maximum Contaminant Level, or MCL) and diazinon was tested in the 7.7 to 29.7 ppb range. An atrazine concentration as high as 10 ppb -- 3 times higher than the MCL -- showed no effect on diazinon's EC50. Additional cited studies in the document discuss the joint toxicity of atrazine and diazinon as measured in 96 hour static toxicity tests of a small shrimp like amphiphod (*Hyallella azteca*) and, separately, in the common housefly (*Musca domestica*). In general, we believe that evidence linking atrazine exposure with potentiation of diazinon toxicity is limited at best, not directly related to species of interest, and -- if present -- occur only at concentrations far higher than those associated with actual drinking water sources. Thus, we believe that the potentiating effects of atrazine on the toxicity of diazinon have not been demonstrated at concentrations relevant to human health.

Response 3
Part A - Reliance on non-mammalian studies
BINWOE methodology. As stated in the interaction profile, the weight-of-evidence approach of binary combinations is used to evaluate the overall toxicity of the mixture; i.e., the evaluation provides important "qualitative" information on the predominant

direction of all interactions (additivity, more-than additivity, less than additivity). The methodology is described in the ATSDR's *Guidance Manual for Assessment of Joint Toxic Action of Chemical Mixtures* (www.atsdr.cdc.gov). This document underwent rigorous peer-reviews and public reviews and was endorsed by scientists from governmental agencies in the U.S.A. (EPA, NIEHS) and Europe (Health Council of the Netherlands). It outlines ATSDR's strategy for exposure-based assessment of joint toxic action of chemicals and the decision process (in flow-charts) that is to be followed in cases when pertinent data are missing or insufficient.

The U.S. EPA has developed a similar approach (*Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures*. EPA 2000). Both agencies prefer human data; however, if human data are not available, studies in animals *in vivo* or studies *in vitro* systems can be used to assess the joint toxic action. Weighting factors and categorizations for mechanistic understanding and toxicological significance reflect the overall confidence in the binary weight-of-evidence derivation (EPA 2000, ATSDR 2004).

As explained in the interaction profile: "Diazinon is a phosphorothioate organophosphorus insecticide that is metabolically activated through oxidative desulfuration to diazoxon by cytochrome P450. Diazoxon binds to acetylcholinesterase, inhibiting its ability to hydrolyze the neurotransmitter acetylcholine. The resulting **accumulation of acetylcholine at the nerve endings causes continual neurological stimulation. This mechanism of action applies to both invertebrates and mammals.** Atrazine induced the metabolic activation of a similar phosphorothioate organophosphorus insecticide, chlorpyrifos, and potentiated its acute neurotoxicity to midges (Belden and Lydy 2000). Based on the similarity in structure and mechanism of action of diazinon and chlorpyrifos, **a similar mechanism** (induction of metabolic activation) **can be inferred for atrazine's potentiation of the acute neurotoxicity of diazinon** to midges in the same study. Because the mechanism of interaction is inferred from a similar chemical, a rating of II is chosen for mechanistic understanding." Therefore, based on the approved BINWOE methodology, the use of non-mammalian species is adequate.

Independent external peer-reviewers of interaction profiles consist of experts in the field of chemical mixtures. The reviewers' comments on this profile also confirmed ATSDR's evaluations. One of the reviewers pointed out regarding the atrazine/deethylatrazine on diazinon interaction that "[t]he arguments "one relevant study" and "insect-human differences" are irrelevant here. Where the dose levels are appropriate, it is clear that such a potentiation will occur – that is the inference from the study with related chemicals." Further, the reviewer stated regarding the simazine on diazinon interaction that "in this case, the toxicological consequences can be inferred from atrazine-chlorpyriphos combination. The arguments of one relevant study and insect-human differences are not relevant to the choice of the score per information presented."

Part B - Concentrations in drinking water sources

For an evaluation of joint toxic action of binary combinations of chemicals (i.e., BINWOE derivation), it is not relevant that the chemicals were found at levels below "their established standards" or below "concentrations found in drinking water" (or other environmental media). For well-studied binary mixtures, the data may suggest no interactions at low doses (joint action appears additive), but interactions at higher doses. In general, the rating should reflect the interaction (ATSDR 2004).
Further, a number of studies indicate co-exposure to subthreshold doses or environmental doses of chemicals that affect the same target organs (though not necessarily by the same mechanism) can result in adverse effects. A mixture of eight xenoestrogens produced significant effects in a recombinant yeast estrogen screen when the individual components were present at below their no-effect concentrations (Silva et al. 2002). An acute study of a mixture of subthreshold doses of 1,1,1-trichloroethane, trichloroethylene, and tetrachloroethylene in rats resulted in adverse effects on the liver; similar results were obtained in hepatocytes *in vitro* (Stacey 1989). Although cadmium and lead affect the hematological system through different mechanisms, dietary exposures of rats to these metals at doses that did not significantly affect hemoglobin and hematocrit when given individually, resulted in significant decreases in hemoglobin and hematocrit when given as a mixture (Mahaffey and Fowler 1977; Mahaffey et al. 1981). A series of studies initiated by the NIEHS on a mixture of 25 groundwater contaminants from hazardous waste sites and on a mixture of pesticide and fertilizer contaminants indicated that toxic effects can result from long-term exposure to mixtures in which each of the components is present at doses expected to be subtoxic (Kligerman et al. 1993; Yang 1994). Epidemiological studies of children have indicated that lead and arsenic, and lead and cadmium, may interact at environmental levels of exposure to produce adverse neurobehavioral consequences in children (Marlowe et al. 1985; Moon et al. 1985).

A consideration of the actual dose received by exposed populations comes with the calculation of the hazard index - a "quantitative" part of the evaluation process. Dose additivity is the underlying assumption of the hazard index method used in the interaction profile. The method is relevant to environmental exposures. In the low-dose region in which dose-response regressions may be linear, which is assumed in absence of data to contrary, dose additivity may hold even for components with different (i.e., independent) mechanisms (EPA 1986, 1990, 2000).

Comment 4
<u>Potentiating effects of atrazine on the toxicity of nitrate</u>.

The DIP also discusses potential for chemical interaction between atrazine and nitrite (a metabolite of nitrate) and hypothesizes that these may form N-nitrosoatrazine. The document states that "the formation of N-nitrosamines from pesticide amino groups and nitrite is of concern because most N-nitrosamines are carcinogenic." The document then reviews several *in vitro* studies in which the formation of N-nitrosamines has been demonstrated during incubation with human gastric juice at 37 C. In *in vivo* studies with mice gavaged with 1000 ug atrazine or 500 ug atrazine followed by 500 ug nitrite, a small amount of conversion of atrazine to N-nitrosoatrazine was seen in some or all the mice. We note that gavage doses of atrazine of this magnitude are extremely high and far

exceed that which would be expected in humans through drinking water. Specifically, even at concentrations of 100 ppb atrazine in water, an individual would need to consume 5 L of water to ingest a dose of atrazine equivalent to those dosed in the mice. At a still high-end atrazine concentration of 10 ppb -- more than 3 times higher than the EPA MCL for atrazine and far higher than is generally seen in even high-end drinking water systems -- this would translate to a consumption of 50 L of water. Further, the cited studies showed no conversion of atrazine to N-nitrosoatrazine at the lowest dose tested, 250 ug atrazine and 500 ug nitrite.

Response 4

Review of the literature

As indicated in the interaction profile, a thorough review of the current literature indicated that there is a possibility of nitrosoatrazine and nitrososimazine formation *in vivo*. As explained in the response to comment number 3, environmental doses are not considered when evaluating binary interactions. However, they are considered in the final evaluation in which exposures are included in the HI calculations and also further when recommendations relevant to public are made.

QSAR model

ATSDR's interagency workgroup met on April 24, 2003 to discuss the binary weight-of-evidence determinations in the *Interaction Profile for Atrazine, Deethylatrazine, Diazinon, Nitrate and Simazine*. EPA representative (one of the authors/contributors of the *EPA's Guidance for Conducting Risk Assessment of Chemical Mixtures*) was present at the meeting. The workgroup recommended performing the SAR (structure activity relationship) analyses on N-nitrosoatrazine and N-nitrososimazine. The SAR analyses were performed using the TOPKAT software. TOPKAT is a desk-top computer based model that predicts the toxicity of chemicals based on their molecular structure. It compares the structure of the queried chemical to chemical structures of experimentally tested chemicals stored in the database and predicts the probability of an effect. For the lack of data in the database, the TOPKAT did not consider the nitrosamine substructure itself, but did consider nitro and N-N structures. Carcinogenicity results were inconclusive. For example, carcinogenicity was predicted with low confidence in male rats for N-nitrosoatrazine and with high confidence in male rats for N-nitrososimazine. In contrast, non-carcinogenicity with low confidence was predicted in male mouse for both compounds. Possible decrease in carcinogenic activity of these compounds as compared with other nitrosoamines may be based on steric hindrance and bulky substituents. In contrast, the MULTICASE software conclusion predicted that both compounds would be carcinogenic (92%). In addition, predictions of carcinogenicity for both compounds were done again in March 2006 using DEREK (Lhasa, Ltd.) software. Carcinogenicity in mammals for N-nitrosoatrazin and N-nitrososimazine was predicted as plausible, based on the presence of "secondary amine", "N-nitro or nitroso compound", and "aromatic amine."

ATSDR agrees to add a statement reflecting EPA's summary that the issue of atrazine/nitrate combination and potential human cancer risk is still unresolved.

This statement will guide the health assessors making predictions on the toxicity of the whole mixture.

Comment 5

We are aware of a study conducted that is not mentioned in the DIP but we think is as or more relevant than the study cited above. In this study, the authors found that N-nitrosoatrazine is readily formed from atrazine and nitrite at acid pH and is mutagenic in the Ames and Chinese Hamster V-79 assays (see Wiesenburger, D.D. (1987) and Wiesenburger, D. D. (1988)). As follow-on work, the authors performed carcinogenesis tests in 250 female Swiss mice and 250 female Wistar rats treated in five groups as follow: (i) with atrazine at the maximum tolerated dose of 1500 ppm in mice and 500 ppm in rats; (ii) sodium nitrite in drinking water *ad libitum* at 3 g/L (3000 ppm); (iii) atrazine + sodium nitrate (as above); (iv) N-nitrosoatrazine by gavage twice weekly at 1/20 the LD50, or 65 mg/kg in mice and 175 mg/kg in rats; and (v) untreated. Although the doses of atrazine were decreased over time in both species due to excessive toxicity and all treatments were discontinued at 67 weeks, no significant increases in tumors were found in any of the treatment groups and the authors concluded that atrazine and N-nitrosoatrazine were not carcinogenic in the species tested. We believe that the *in vivo* carcinogenicity studies performed by the authors are more relevant to the human exposures of interest here than the studies currently cited in the draft DIP and should be given appropriate mention and consideration in any revised DIP.

Response 5

ATSDR is aware of the Wiesenburger 1987 and 1988 studies and they were listed in the reference list of this interaction profile. However, the studies were published only in the form of short abstracts in the *Proceedings of American Association for Cancer Research* and ATSDR generally does not cite studies that have been published only as abstracts, because of concerns regarding study quality. A current abstract can be referenced only if the original paper is not obtainable. Older abstracts should be disregarded if not followed up in the literature. Specifically, the Guidance for the Preparation of an Interaction Profile (ATSDR 2004) instructs the writers: “Current abstracts should be discussed in the "Ongoing Studies" sections of Chapters 2, 5, and 6. In almost all cases, abstracts should be disregarded if not followed up in the literature. Citation of an abstract in a profile requires the ATSDR chemical manager's approval.”

In accordance with this policy, ATSDR’s chemical manager contacted the principal author, Dr. Wiesenburger, in 2003 when the interaction profile was drafted. Phone conversations with Dr. Wiesenburger made it clear that he did not publish a full paper with the results presented in the abstract and he does not intend to do so in the future. When asked about the raw data that ATSDR would be willing to submit for an external peer-review, Dr. Wiesenburger indicated that the studies were done long time ago, he does not know about the original data and referred ATSDR to his colleague that may possibly find the data. However, that colleague was unresponsive to ATSDR’s inquiries. Because ATSDR was not able to verify the studies, a decision was made not to include them in the interaction profile.

Comment 6
An epidemiological study investigating cancer rates and drinking water containing atrazine (0.050 to 0.649 ppb) and nitrate (at concentrations up to 91 mg/L) is also discussed in the document. The DIP, we believe, offers appropriate cautions with respect to the study design, indicates that it does not establish causality, is not supported by other studies of atrazine or nitrate, and does not provide suggestive evidence of a greater-than-additive interaction since no cancer type was positively correlated with both atrazine and nitrate concentrations. In sum, then, we believe that there is insufficient evidence to associate combined atrazine + nitrate exposure at environmentally relevant concentrations with increased cancer risks and a balanced discussion of the evidence and its limitations is needed: we recommend that a specific statement be made that the issue of nitrate and potential human cancer risk is unresolved.

Response 6
See response to comment number 4. ATSDR agrees to add a statement reflecting EPA's summary that the issue of atrazine/nitrate combination and potential human cancer risk is still unresolved. This statement will guide the health assessors making predictions on the toxicity of the whole mixture.

Comment 7
The DIP also discussed a number of other studies involving the joint action of atrazine and nitrate on northern leopard frog (*Rana pipiens*) larvae and newt larvae. None of these species are particularly or directly relevant to the doses or exposure pathways that would apply to humans.

Response 7
These studies are cited along with studies in mice and serve to show consistent results across species.

Comment 8
<u>Potentiating effects of simazine on the toxicity of nitrate</u>.

As described above in relation to atrazine and nitrate, the DIP states that the formation of N-nitrosamines from pesticide amino groups and nitrate is of concern because most N-nitrosamines are carcinogenic. The document states that simazine and nitrite were shown to react at acidic pH to form N-nitrososimazine.

The DIP cites a study in which gavage administration of 2.3 mg/kg of radiolabeled simazine and sodium nitrite at 20.5 mg/kg resulted in an increase in labeled N-nitrososimazine in the liver and thymus relative to the amounts formed from simazine alone at the same dose in the mixture. Increases in other organs (kidney and spleen) were not significantly statistically different from those seen following administration of simazine alone. As with the case of atrazine, the simazine doses are far in excess of what would normally be seen in drinking water bodies or ingested. A dose of 2.3 mg/kg would equate to a concentration of 23 ppm using standard EPA default body weight and

drinking water ingestion rates. This is several orders of magnitude greater than those observed in even the most contaminated areas in the U.S.

Response 8
See response to comment 4.