January 10, 2006

MEMORANDUM

Subject: **Comments** on ATSDR Draft Interaction Profiles:
            "*Interaction Profile for : Atrazine, Deethylatrazine, Diazinon, Nitrate, and Simazine*"
                                                and
            "*Interaction Profile for Chlorpyrifos, Lead, Mercury, and Methylmercury*"

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Thank you very much for the opportunity to review the ATSDR draft interaction profiles for (i) atrazine, deethylatrazine, diazinon, nitrate, and simazine; and (ii) chlorpyrifos, lead, mercury, and methylmercury.   EPA and ATSDR have long had a productive and mutually-beneficial relationship that has permitted both our Agency's to assist in advancing the science of public health protection.

We understand ATSDR has initiated a mixtures program to identify mixtures most often found in environmental media and to perform quantitative modeling and methodological development of mixture interactions and joint toxicity. We also understand that as part of this mixtures program ATSDR publishes a series of "Interaction Profiles" for certain priority mixtures that are of special concern to ATSDR. These interaction profiles

evaluate data on the toxicology of the whole priority mixture and on the joint toxic action (including antagonism and synergism) of the chemicals in the mixture.

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.

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.[1] 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:

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

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To carry out the legislative mandate under CERCLA, ATSDR's
Division of Toxicology …
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[1] In these other contexts, EPA has defined cumulative risk assessment in a broader manner: Specifically, "the examination of the *accumulation* (over time, across sources, across routes) of stressors or exposures that can cause adverse effects, and then the *integration* of the effects these stressors or exposures cause into an estimated characterization of the risk caused to the individual or population by the stressors *acting together"* (US EPA, 2002)   We believe that this alternate definition is more in line with the <u>*interactions*</u> that ATSDR is considering in its Draft Interaction Profiles.

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:

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

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 (*Chironomus 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.

Potentiating effects of atrazine on the toxicity of nitrate.

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 have 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

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cited studies showed no conversion of atrazine to N-nitrosoatrazine at the lowest dose tested, 250 ug atrazine and 500 ug nitrite.

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.[2] 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.

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.

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.

Potentiating effects of simazine on the toxicity of nitrate.

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-

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[2] The authors did, however, recommend that additional studies be conducted in hamsters: they found in subsequent studies that N-nitrosoatrazine is mutagenic in the Ames assay using hamster liver S9 activation, but is not mutagenic when rat liver S9 fraction is used.

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.[3] 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.

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[3] The document does not indicate which species this test was performed in.