INTERACTION PROFILE FOR:
ATRAZINE, DEETHYLATRAZINE, DIAZINON, NITRATE, AND SIMAZINE

U.S. Department of Health and Human Services
Public Health Service
Agency for Toxic Substances and Disease Registry

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PREFACE

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) mandates that the Agency for Toxic Substances and Disease Registry (ATSDR) shall assess whether adequate information on health effects is available for the priority hazardous substances. Where such information is not available or under development, ATSDR shall, in cooperation with the National Toxicology Program, initiate a program of research to determine these health effects. The Act further directs that where feasible, ATSDR shall develop methods to determine the health effects of substances in combination with other substances with which they are commonly found.

To carry out the legislative mandate, ATSDR's Division of Toxicology and Environmental Medicine (DTEM) has developed and coordinated a mixtures program that includes trend analysis to identify the mixtures most often found in environmental media, *in vivo* and *in vitro* toxicological testing of mixtures, quantitative modeling of joint action, and methodological development for assessment of joint toxicity. These efforts are interrelated. For example, the trend analysis suggests mixtures of concern for which assessments need to be conducted. If data are not available, further research is recommended. The data thus generated often contribute to the design, calibration or validation of the methodology. This pragmatic approach allows identification of pertinent issues and their resolution as well as enhancement of our understanding of the mechanisms of joint toxic action. All the information obtained is thus used to enhance existing or developing methods to assess the joint toxic action of environmental chemicals. Over a number of years, ATSDR scientists in collaboration with mixtures risk assessors and laboratory scientists have developed approaches for the assessment of the joint toxic action of chemical mixtures. As part of the mixtures program a series of documents, Interaction Profiles, are being developed for certain priority mixtures that are of special concern to ATSDR.

The purpose of an Interaction Profile is to evaluate data on the toxicology of the "whole" priority mixture (if available) and on the joint toxic action of the chemicals in the mixture in order to recommend approaches for the exposure-based assessment of the potential hazard to public health. Joint toxic action includes additivity and interactions. A weight-of-evidence approach is commonly used in these documents to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although ATSDR recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur.

ATSDR will use the following process for the development of interaction profiles:

- ATSDR will select substances/chemicals for development of interaction profiles through inter/intra agency communications and literature reviews.
- After the selection, a letter will be sent to individuals and agencies on ATSDR's mailing list providing notice of ATSDR's intent to create an interaction profile.
- A notice will also be posted in the Federal Register to inform the public of ATSDR's intent to develop a particular interaction profile.
- The draft interaction profile will undergo both internal and external peer review processes.
- A Federal Register notice will announce the release of the official draft for public comment.
- ATSDR will post a link to the draft interaction profile on its Website, giving the public an opportunity to provide comments.
- ATSDR will review all public comments and revise the draft, as appropriate, before issuing the final version.

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PEER REVIEW

A peer review panel was assembled for this profile. The panel consisted of the following members:

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All reviewers were selected in conformity with the conditions for peer review specified in CERCLA Section 104(I)(13).

Scientists from ATSDR have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

SUMMARY

Atrazine, deethylatrazine, simazine, diazinon, and nitrate were chosen as the subject mixture for this interaction profile because they frequently occur together in rural well water. Atrazine and simazine are triazine herbicides, deethylatrazine is a metabolite and an environmental degradation product of atrazine and other triazine herbicides, diazinon is an organophosphorus insecticide, and nitrate is a common contaminant resulting from fertilizers and human and animal waste. The exposures of greatest concern for this mixture in rural well water are intermediate and chronic oral exposures. No pertinent health effects data or physiologically-based pharmacokinetic (PBPK) models were located for the complete mixture. Therefore, the exposure-based screening assessment of potential health hazards for this mixture depends on an evaluation of the health effects and mechanistic data for the individual components and on the joint toxic action and mechanistic data for various combinations of the components. This profile discusses and evaluates the evidence for joint toxic action among atrazine, deethylatrazine, simazine, diazinon, and nitrate. The profile also discusses how public health assessments can incorporate concerns about interactions, additivity, and potential human exposures to mixtures of these chemicals.

Effects of concern for this mixture include reproductive effects (atrazine, deethylatrazine, and simazine), neurological effects (diazinon), and hematological effects (nitrate). Although none of the components has been classified as a carcinogen, atrazine and simazine can react with nitrite (nitrate metabolite) in the environment and *in vivo* to form N-nitrosoatrazine and N-nitrososimazine. Structure-activity considerations raise a concern for potential carcinogenicity of these nitrosamines. However, the issue of atrazine/nitrate and simazine/nitrate combinations and potential cancer risk in humans is still unresolved and further research is needed.

To screen the mixture of atrazine, deethylatrazine, simazine, diazinon, and nitrate for potential hazards to public health, the hazard quotients (ratios of exposures to health guidance values) are estimated for the individual components. If only one or if none of the components has a hazard quotient that is at least 0.1, no further assessment of the *joint toxic action* is needed because additivity and/or interactions are unlikely to result in significant health hazard. If the hazard quotients for two or more of the mixture components equal or exceed 0.1, the following procedures are recommended. To screen this mixture for potential reproductive health hazard, an endpoint-specific hazard index for reproductive effects should be estimated for atrazine, deethylatrazine, and simazine (the triazine components of the mixture). The weight-of-evidence (WOE) analysis for interactions among these components indicates high confidence in the additivity assumption that is the basis for the hazard index. The potential effect of diazinon and nitrate on

the reproductive toxicity of these triazines is uncertain. Separate hazard quotients are recommended to screen for the neurotoxicity of diazinon and the hematological toxicity of nitrate. The WOE analysis indicates that because the triazine components may potentiate the neurologic toxicity of diazinon, the hazard quotient for diazinon may tend to underestimate the hazard of exposure to diazinon when these triazine components are present. Confidence in these predictions is medium. No information regarding the impact of interactions on the hematological toxicity of nitrate was available, so uncertainty regarding the impact of the other components on this effect of nitrate is high.

If the reproductive hazard index for the triazines or the hazard quotient for nitrate is greater than 1, or if the hazard quotient for diazinon is close to or above 1, then further evaluation is needed (ATSDR 2001a), using biomedical judgment and community-specific health outcome data.

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ATSDR	Agency for Toxic Substances and Disease Registry	NRC	Nuclear Regulatory Commission
BINWOE	binary weight-of-evidence	NTP	National Toxicology Program
CAS	Chemical Abstracts Service	PAD	population adjusted dose
CERCLA	Comprehensive Environmental Response, Compensation, and Recovery Act	2-PAM	pralidoxime
CHO	Chinese hamster ovary	PBPK	physiologically based pharmacokinetic
DACT	diaminochlorotriazine	PBPK/PD	physiologically-based pharmacokinetic/pharmacodynamic
DT	Division of Toxicology	ppb	parts per billion
EC_{50}	median effective concentration (produces desired effect in 50% of population)	RfC	reference concentration
EPA	Environmental Protection Agency	RfD	reference dose transaminase
FQPA	Food Quality Protection Act	SMR	standardized mortality ratio
GnRh	gonadotropin releasing hormone	TTD	target-organ toxicity dose
IARC	International Agency for Research on Cancer	µg	microgram
IRIS	Integrated Risk Information System	µmole	micromole
kg	kilogram	U.S.	United States
L	liter	VOC	volatile organic compound
LC_{50}	median lethal concentration (produces desired effect in 50% of the population)	WOE	weight-of-evidence
LH	luteinizing hormone		
LOAEL	lowest-observed-adverse-effect level	>	greater than
MCL	maximum contaminant level	≥	greater than or equal to
MCLG	maximum contaminant level goal	=	equal to
mg	milligram	<	less than
mM	millimole	≤	less than or equal to
MRL	Minimal Risk Level		
NADH	nicotinamide adenine dinucleotide phosphate		
ng	nanogram		
NOAEL	no-observed-adverse-effect level		

1. Introduction

The primary purpose of this Interaction Profile for atrazine, deethylatrazine, diazinon, nitrate, and simazine is to evaluate data on the toxicology of the "whole" mixture and the joint toxic action of the chemicals in the mixture in order to recommend approaches for assessing the potential hazard of this mixture to public health. To this end, the profile evaluates the whole mixture data (if available), focusing on the identification of health effects of concern, adequacy of the data as the basis for a mixture Minimal Risk Level (MRL), and adequacy and relevance of physiologically-based pharmacokinetic/ pharmacodynamic (PBPK/PD) models for the mixture. The profile also evaluates the evidence for joint toxic action—additivity and interactions—among the mixture components. A weight-of-evidence (WOE) approach is commonly used in these profiles to evaluate the influence of interactions in the overall toxicity of the mixture. The weight-of-evidence evaluations are qualitative in nature, although the Agency for Toxic Substances and Disease Registry (ATSDR) recognizes that observations of toxicological interactions depend greatly on exposure doses and that some interactions appear to have thresholds. Thus, the interactions are evaluated in a qualitative manner to provide a sense of what influence the interactions may have when they do occur. The profile provides environmental health scientists with ATSDR Division of Toxicology and Environmental Medicine's (DTEM) recommended approaches for the incorporation of the whole mixture data or the concerns for additivity and interactions into an assessment of the potential hazard of this mixture to public health. These approaches can then be used with specific exposure data from hazardous waste sites or other exposure scenarios.

The atrazine, deethylatrazine, diazinon, nitrate, and simazine mixture was chosen as the subject for this interaction profile based on analyses of frequently occurring mixtures in groundwater. As part of the National Water-Quality Assessment Program of the U.S. Geological Survey, untreated groundwater samples were collected from 1,255 domestic (rural) wells and 242 public water-supply wells, and analyzed for 60 volatile organic compounds (VOCs), 83 pesticides, and nitrate (Squillace et al. 2002). The most frequently occurring four-chemical mixture in these groundwater samples consisted of two triazine herbicides and a metabolite (atrazine, simazine, and deethylatrazine), plus nitrate. Concentrations of the 144 monitored chemicals were screened against drinking water standards and health advisories. Nitrate was the chemical that most frequently exceeded its standard or criterion (maximum contaminant level [MCL] for nitrate = 10 mg/L as nitrogen). Atrazine and simazine did not exceed their MCLs (0.003 and 0.004 mg/L, respectively). Diazinon was the most frequently detected organophosphorus insecticide, and exceeded its drinking water health advisory (0.0006 mg/L) in one well. Diazinon was selected for the mixture in order to evaluate possible interactions of organophosphates with other

pesticides. The primary route of exposure for this mixture is likely to be oral and the durations of concern are intermediate and chronic.

Before evaluating the relevance of joint toxic action data for these chemicals, some understanding of endpoints of concern for oral exposure to this mixture is needed. The endpoints of concern include the critical effects that are the bases for MRLs or other health guidance values, and any other endpoints that may become significant because they are shared targets of toxicity or due to interactions (ATSDR 2001a).

In order to satisfy the requirements of the Food Quality Protection Act (FQPA) to assess the cumulative effects of chemicals that have a common mechanism of toxicity, certain triazine herbicides, including atrazine, its metabolite deethylatrazine (also known as desethylatrazine, desethyl s-triazine), and simazine, are being reevaluated by the Environmental Protection Agency's (EPA) (2002c) Office of Pesticide Programs. The EPA (2002c) has concluded that these triazines should be considered as a *Common Mechanism Group* based on suppression of the luteinizing hormone ovulatory surge and the resulting effects on reproductive function and reproductive development. EPA (2002b) has derived a new chronic reference dose (RfD) for atrazine and its chlorinated metabolites, including deethylatrazine, based on reproductive effects; this RfD is not on the Integrated Risk Information System (IRIS) (2003), but its derivation includes a consideration of mechanistic and toxicological data that have become available since the RfD on IRIS was derived. EPA has not yet derived a new RfD for simazine. Further explanation is provided in Appendices A and B. ATSDR (2003) evaluated atrazine in a toxicological profile. ATSDR (2003) derived an intermediate oral MRL of 0.003 mg/kg/day based on reproductive effects (delayed onset of estrus) in pigs. Thus, reproductive effects are the effects of concern for atrazine, deethylatrazine, and simazine.

Diazinon's critical effect, which is the basis of ATSDR (1996) MRLs and EPA (2000; IRIS 2003) RfDs, is neurological, due to inhibition of acetylcholinesterase. Nitrate, through reduction to nitrite, causes methemoglobinemia, which is the critical effect for EPA's (IRIS 2003) RfD.

None of these chemicals has been classified as a carcinogen (see Appendices), but a chemical interaction between atrazine and nitrite and between simazine and nitrite results in the formation of N-nitrosoatrazine and N-nitrososimazine. These nitrosamines have not been adequately tested for carcinogenicity, but structure-activity considerations raise a concern that they may have carcinogenic potential.

Thus, the endpoints of concern for this mixture are reproductive, neurological, hematological, and carcinogenic. The structures and the Chemical Abstracts Service (CAS) Registry Numbers of these chemicals are provided in Appendix E.

2. Joint Toxic Action Data for the Mixture of Concern and Component Mixtures

This chapter provides a review and evaluation of the literature pertinent to joint toxic action of the mixture and its components.

2.1 Mixture of Concern

Toxicological data or PBPK models were not available for the complete mixture of concern.

2.2 Component Mixtures

Toxicological and mechanistic data, but no PBPK models, were available for some of the binary mixtures. With the exception of the data for the joint action of the triazines, these data were fairly limited. Atrazine and deethylatrazine are generally considered together as one component in this profile, because of the similarity in their metabolism and mechanism of action, and because deethylatrazine is a metabolite and environmental degradation product of atrazine (Appendix A).

2.2.1 Atrazine/Deethylatrazine and Simazine

In a study of neuroendocrine/reproductive effects in mature male Atlantic salmon parr (salmon living in fresh water), short-term exposure of the olfactory epithelium (*in situ*) to atrazine (1.0 µg/L) or simazine (1.0 or 2.0 µg/L) significantly reduced the olfactory response to the female priming pheromone, prostaglandin $F_{2\alpha}$ (Moore and Lower 2001). The response was determined electrophysiologically in anesthetized fish. Exposure to a mixture of the two herbicides as a 1:1 mixture at total concentrations of 1.0 and 2.0 µg/L resulted in reductions that were not significantly different from the single chemicals at the same concentrations. Thus, results indicated concentration (dose) addition. Similar experiments with the individual chemicals and mixtures studied the impact on the reproductive priming effect of prostaglandin F2α on the levels of expressible milt and on plasma levels of testosterone, 11-ketotestos-terone, and 17,20β-dihydroxy-4-pregnen-3-one in unanesthetized fish exposed for 5 days. Results indicated additivity with regard to a reduction in expressible milt and on hormonal status.

Atrazine and simazine were tested for concentration addition in green algae, using the inhibition of reproduction of synchronized cultures of *Chlorella fusca* during one generation as the endpoint (Faust et al. 1993). The observed median effective concentration (EC50) of the mixture was virtually the same

as that predicted on the basis of concentration addition. This result was expected because both herbicides inhibit photosystem II. The experimental design was adequate to support this conclusion, but the relevance to human health is questionable.

A study in Chinese hamster ovary (CHO) cells incubated with atrazine and/or simazine used the coefficient of variation of the G1 peaks (nuclei) and of the largest chromosome peak (isolated chromosomes) as indices of clastogenicity (Taets et al. 1998). Chromosome breakage causes uneven distribution of DNA in nuclei of daughter cells, increasing the variability in nuclear size and hence the coefficient of variation; a similar impact is seen on chromosome size and variability. When tested at the levels of EPA MCLs (0.003 mg/L atrazine, 0.001 mg/L simazine, or mixture of 0.003 mg atrazine plus 0.001 mg/L simazine), the coefficient of variation for the G1 peaks was significantly elevated to a similar extent for both herbicides individually and for the mixture as compared with controls. Similar results were seen for the coefficient of variation for the largest chromosome peak, but the increase for simazine alone was not statistically significant. When tested at the highest levels found in Illinois water supplies (0.018 mg/L atrazine, 0.004 mg/L simazine, or mixture of 0.018 mg/L atrazine plus 0.004 mg/L simazine), similar results were found for G1 peaks. For the largest chromosome peak, however, results are uncertain because the description of the results for atrazine in the text, the table, and the figure are not consistent. Limitations of this study include lack of statistical comparison of results from the mixtures with the single chemicals, higher combined dose of chemicals in the mixture than in the single chemical treatments, and the inconsistent reporting of results for atrazine. Under dose addition, a higher degree of clastogenicity would be expected from the mixtures as compared with the single chemicals in this study, but the higher combined dose in the mixture groups may have been more cytotoxic. Cytotoxicity, according to the study authors, would tend to result in selection for resistant cell types that are more homogeneous, which would lower the coefficient of variation. Thus, the study design and results are inadequate to support meaningful conclusions regarding the type of joint action.

Neither atrazine nor simazine nor the mixture of the two produced a mutagenic response in *Salmonella typhimurium* TA1535, TA1537, TA1538, TA98, or TA100 with or without a rat liver S-9 activating system (Eisenbeis et al. 1981). A range of concentrations was tested from ‘full strength’ down to zero; details were not provided.

Analysis of studies of mode of action of certain triazine herbicides, including atrazine and simazine, and their chlorinated metabolites, including deethylatrazine, has indicated that they have a common mechanism of toxicity with regard to attenuation of the luteinizing hormone (LH) surge in female and

male rats, alteration of the estrous cycle, delayed pubertal development in both sexes of rats, and altered pregnancy maintenance (EPA 2002c). These triazines are not estrogenic. Rather, their mechanism of reproductive toxicity involves neuroendocrine disruption of hypothalamic-pituitary-gonadal function. In female Sprague-Dawley rats, atrazine accelerates the normal process of reproductive sensescence, which is initiated by a failure of the hypothalamus to release levels of gonadotropin releasing hormone (GnRH) that are adequate to stimulate the pituitary to release LH. Without sufficient LH, ovulation does not occur, estrogen levels remain high, persistent estrus occurs, and mammary gland tumors develop. In other strains of rats, atrazine causes elevated progesterone levels, which leads to pseudopregnancy and persistent diestrus, but not mammary tumors. The carcinogenic outcome is not expected in humans, due to species and strain differences in reproductive senescence. Reproductive senescence in female Sprague-Dawley rats involves decreasing hypothalamic function and *increased* serum estrogen levels (thought to contribute to mammary gland cancer), whereas reproductive senescence in women involves ovarian depletion and *decreased* serum estrogen levels (ATSDR 2003; EPA 2002a, 2002b, 2002c). Further mechanistic detail is provided in Section A.3 of Appendix A. Although the carcinogenicity of these triazines in female Sprague-Dawley rats is not thought to be applicable to humans, the neuroendocrine disruption at the level of the hypothalamus, resulting in altered hypothalamic-pituitary function, is considered to be relevant to humans. The mode of action of atrazine, deethylatrazine, and simazine with regard to reproductive function and reproductive development is expected to be dose additive (EPA 2002c).

2.2.2 Atrazine and Diazinon

No studies of this binary mixture in mammals were located. A study on the joint toxic action of diazinon and atrazine in midge (*Chirononus tentans*) larvae reported that environmentally relevant concentrations of atrazine (40–200 µg/L) potentiated the acute neurotoxicity of diazinon (7.7–29.7 µg/L) in 96-hour static toxicity tests (Belden and Lydy 2000). Acute neurotoxicity was measured as the inability of the midges to perform normal swimming motions. Based on changes in the EC50 values, atrazine treatment at 40, 80, and 200 µg/L increased the toxicity of diazinon 1.81-, 2.11-, and 2.71-fold, respectively. No effect on the diazinon EC50 was seen at 10 µg/L atrazine. Atrazine alone was not acutely toxic to midges even at the limit of water solubility (10,000 µg/L), which was 50 times the highest atrazine concentration used in the study. The effect of atrazine on diazinon toxicity may have been mediated through induction of cytochrome P450 enzymes that activate organophosphorus insecticides. This conclusion is based on induction of the metabolism and potentiation of the neurotoxicity of another organophosphorus insecticide, chlorpyrifos, by atrazine in additional experiments in this study.

Additional studies of joint toxic action of atrazine and diazinon in an aquatic amphipod (*Hyalella azteca*, a small shrimp-like creature) also reported potentiation of diazinon toxicity by atrazine (Anderson and Lydy 2002). In 96-hour static toxicity (median lethal concentration [LC50]) assays, atrazine at 80 and 200 μg/L increased the acute toxicity of diazinon 2.0- and 3.0-fold, respectively. No effect on the diazinon LC50 was seen at ≤40 μg/L atrazine. Atrazine alone was not lethal at a concentration of 10,000 μg/L. In additional studies on acetylcholinesterase activity, atrazine alone (200 μg/L) had no effect during 96-hour static tests. At the 96-hour LC01 for diazinon (0.90 μg/L), in comparison with controls, acetylcholinesterase activity was 27% lower with diazinon alone, and 43% lower with diazinon and atrazine (200 μg/L), indicating a potentiation by atrazine of diazinon toxicity.

In the common house fly (*Musca domestica*), however, atrazine (200 or 2,000 ng/mg body weight = 0.2 or 2 μg/mg body weight) did not affect the acute lethality of diazinon (0.2–9.9 ng/mg body weight), when both chemicals were applied in acetone to the ventral abdomen (Anderson and Lydy 2002). To test whether the atrazine penetrated the cuticle, a much smaller dose of radiolabeled atrazine (1.27 ng/mg body weight) was applied to the ventral abdomen in the same volume of acetone as for the joint action study; radioactivity did appear to penetrate the cuticle. This experiment did not demonstrate whether or not atrazine was actually absorbed by the flies. The studies in midge larvae and in amphipods indicate that potentiation may not be seen at low doses of atrazine. Thus, it is unclear whether the lack of potentiation by atrazine in flies represents a species difference, or whether the atrazine dose actually absorbed by the flies was too low to be effective. Oral administration of atrazine may result in a higher internal dose, but was not tested.

Diazinon is metabolically activated by cytochrome P450 to diazoxon, which binds to acetylcholinesterase, inhibiting the ability of this enzyme to hydrolyze acetylcholine, a neurotransmitter. This inhibition results in continued neurological stimulation. Acetylcholinesterase inhibition is the principal toxic effect in humans and animals, including insects. Thus, the results in the studies in midges and amphipods may be applicable to humans, and indicate greater-than-additive influence of atrazine on diazinon neurotoxicity.

2.2.3 Simazine and Diazinon

No studies of this binary mixture were located. Data from the atrazine-diazinon mixture, reviewed in the previous section, may be relevant because of the similarities between simazine and atrazine. Reasoning

by analogy with atrazine, the influence of simazine on diazinon neurotoxicity would be expected to be greater than additive.

2.2.4 Atrazine and Nitrate

The potential for a chemical interaction between atrazine and nitrite (the metabolite of nitrate) resulting in the formation of N-nitrosoatrazine has been investigated. The formation of N-nitrosamines from pesticide amino groups and nitrite is of concern because most N-nitrosamines are carcinogenic (Lijinsky 2001; Preussmann and Stewart 1984).

Atrazine and nitrite have been shown to react at acidic pH to form N-nitrosoatrazine (Eisenbrand et al. 1975b; Krull et al. 1980; Mirvish et al. 1991; Wolfe et al. 1976). N-Nitrosoatrazine has been tentatively identified in Mississippi River water and New Orleans drinking water (Fine et al. 1976). No formation of N-nitrosoatrazine was detected in soils adjusted to pHs of 2.5–5.5 and incubated with atrazine and a molar excess of nitrate (limit of detection 10 ppb) for 1–3 months (Kearney et al. 1977). Similar incubation with nitrite, however, resulted in the formation of a small amount of N-nitrosoatrazine at 1 week at pHs of 2.5–5.3, but no nitrosoatrazine was detected at 4 or 10 weeks. Thus, it is unclear whether or not N-nitrosoatrazine could result from nitrate and atrazine in soil, as the initial measurements in that experiment were made after 1 month of incubation. Additional experiments in which N-nitrosoatrazine was added to soil showed that the nitrosamine was degraded (denitrosated to atrazine) (Kearney et al. 1977). N-Nitrosoatrazine was stable in water at 25 °C at pHs above 4 in the dark, but was rapidly decomposed to atrazine and deethylatrazine by light (Wolfe et al. 1976).

The formation of N-nitrosoatrazine from atrazine and nitrite has been demonstrated in human gastric juice (pH 1.5–2.0) during 1.5–12 hours of incubation at 37 °C (Cova et al. 1996). The percent formation peaked at 3 hours, and gradually declined thereafter, due to degradation of N-nitrosoatrazine to atrazine. Peak formation of N-nitrosoatrazine was 2% from 0.05 mM atrazine and 0.5 mM nitrite, 23% from 0.05 mM atrazine and 3 mM nitrite, and 53% from 1 mM atrazine and 3 mM nitrite. The levels of nitrite used were similar to peak gastric levels of nitrite (1.77 mM) in subjects who ingested a salad-type meal containing 1.15 mM of nitrate (Walters et al. 1979).

The formation of N-nitrosoatrazine from atrazine and nitrite also has been demonstrated *in vivo*. Approximately 0.04% conversion occurred within 15 minutes in mice gavaged with 1,000 µg atrazine followed by 500 µg nitrite (Krull et al. 1980). At 500 µg atrazine and 500 µg nitrite, N-nitrosoatrazine

was found in some but not all of the mice, and at 250 µg atrazine and 500 µg nitrite, N-nitrosoatrazine was not detected. The *in vitro* studies conducted as part of this study resulted in conversion of about 0.4% of the atrazine to N-nitrosoatrazine during incubation of 500 µg atrazine with 500 µg nitrate at 37 °C and pH 3 for 2 hours. According to Seiler (1977), the pH of the mouse stomach is approximately 4–5.

A study of cancer rates and drinking water contamination with atrazine (50–649 ng/L) and nitrate (0–91 mg/L) in Ontario "agroecosystems" reported that stomach cancer incidence was positively associated with atrazine concentrations and negatively associated with nitrate concentrations in drinking water (Van Leeuwen et al. 1999). Atrazine concentrations were negatively associated with colon cancer incidence. Associations with other cancer types were not observed. Atrazine and nitrate concentrations in drinking water were positively correlated. The analyses controlled for potential confounding factors such as age and smoking. Limitations of the study include the collection and analysis of data for ecodistricts rather than individuals. In addition, the exposure data were from the same time period as the cancer incidence data. The development of cancer, however, usually involves a latency period, such that previous exposure levels may be more important than concurrent exposure levels. This study does not establish causality, and is not supported by other studies of atrazine or nitrate (see Appendices A and D). Because no cancer type was positively correlated with both atrazine and nitrate concentrations, the study does not provide suggestive evidence of a greater-than-additive interaction as might be expected from nitrosamine formation, but interpretation of the study findings, and particularly the negative correlations, is problematic due to the limitations discussed previously.

The joint toxic action of atrazine and nitrate on northern leopard frog (*Rana pipiens*) larvae was tested (Allran and Karasov 2000). Three concentrations of atrazine (0, 20, and 200 µg/L) and three of nitrate (0, 5, and 20 mg NO_3-N/L) were tested in a factorial design for a total of nine treatments. The selected concentrations bracketed the environmentally relevant range. Neither atrazine nor nitrate nor the mixtures had a significant effect on development rate, growth rate, percent metamorphosis, time to metamorphosis, percent survival, mass at metamorphosis, or hematocrit. Although these results suggest that environmental levels of atrazine and nitrate do not affect the development of the frog, they do not provide useful information on the mode of joint toxic action because the treatments were without effect on the endpoints studied.

The newt (larvae) micronucleus assay gave no indication of clastogenicity for atrazine alone, atrazine with nitrate or nitrite, nitrate alone, and nitrite alone (L'Haridon et al. 1993). Atrazine, nitrate, and nitrite

were tested at levels found in contaminated surface waters, plus nitrate and nitrite were tested at much higher levels. Some of the experiments included preincubation of the mixtures in the dark, to allow for chemical interaction, while minimizing the photodegradation of any N-nitrosoatrazine that might be formed. Testing of N-nitrosoatrazine itself gave slight but statistically significant positive results for clastogenicity at the two highest concentrations. These concentrations (7.5 and 15 ppm, corresponding to 30.6 and 61.2 μM), however, were much higher than could be generated from the concentration of atrazine tested (0.3 ppm, 1.4 μM), even if 100% of the atrazine was converted to the nitrosamine. Thus, if N-nitrosoatrazine is formed from atrazine and nitrate or nitrite in a polluted aquatic environment, the amounts formed may be too low to cause clastogenicity in newt larvae. This study raises concerns for the genotoxicity of N-nitrosoatrazine, but does not provide direct information regarding the mode of joint toxic action of atrazine and nitrate.

Another joint toxic action study used concentrations of atrazine, nitrate, and nitrite alone that were nonclastogenic in human lymphocytes *in vitro*, and half of these non-effective concentrations for testing of the binary mixtures of atrazine and nitrate or atrazine and nitrite (Meisner et al. 1993). No clastogenicity was seen for the mixtures. The concentration of atrazine (0.005 mg/L) in the binary mixtures, while too low to be effective itself, was 5-fold higher than a clastogenic concentration of N-nitrosoatrazine in another experiment reported in the same paper. The yield of N-nitrosoatrazine (if any) under these culture conditions, however, is not known.

A few studies have compared the genotoxicity of atrazine with that of N-nitrosoatrazine. Although these studies did not include investigations of the joint toxic action of atrazine and nitrate or nitrite, they are relevant to the issue of whether the chemical interaction of atrazine and nitrite results in a more toxic chemical, and therefore could be regarded as greater than additive. These studies are summarized below.

Neither atrazine nor N-nitrosoatrazine was mutagenic in *S. typhimurium* TA98, TA100, or TA1537 with or without rat liver S9 (Ishidate 1983; Ishidate et al. 1981), but N-nitrosamines are known to be more readily activated to bacterial mutagens by hamster liver S9 than by rat liver S9 (Lijinsky 2001). Human liver S9 also may be more active than rat liver S9 in N-nitrosamine activation, based on results with a single compound, dimethylnitrosamine (Hakura et al. 2003).

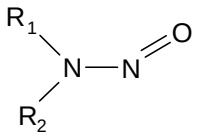
N-nitrosoatrazine was clastogenic in cultured human lymphocytes at concentrations 10,000 times lower than required for atrazine clastogenicity and 1,000 times lower than required for nitrate clastogenicity in the same assay (Meisner et al. 1993). In addition, N-nitrosoatrazine was mitogenic, whereas atrazine and

nitrate were not. In a Chinese hamster cell line derived from lung fibroblasts, N-nitrosoatrazine caused chromosomal aberrations when tested at a concentration 17-fold lower than an atrazine concentration (250 mg/L) that did not cause chromosomal aberrations in the same study (Ishidate 1983; Ishidate et al. 1981). Results of these studies indicate that N-nitrosoatrazine is more clastogenic than atrazine or nitrate, and stimulates cell division whereas atrazine and nitrate do not. This raises a concern that the formation of N-nitrosoatrazine through chemical interaction may be a greater-than-additive interaction in terms of genotoxic and proliferative effects. Implications for carcinogenicity or other effects are less clear.

The metabolism of N-nitrosoatrazine was compared with that of atrazine after oral administration of 50 mg/kg of either chemical to the rat (Meli et al. 1992). The cumulative percentage of the dose of atrazine excreted in the urine as atrazine and metabolites by 96 hours was approximately 37%, whereas for N-nitrosoatrazine, it was approximately 2%. Very little unchanged atrazine and no unchanged N-nitrosoatrazine were detected in urine. The primary urinary metabolite for both compounds was diaminochlorotriazine. *In vitro* studies of the metabolism of atrazine and N-nitrosoatrazine (2 mM of each) in 30-minute incubations with hepatic S9 fractions from untreated rats showed that 37% of the atrazine was metabolized versus 32% of the N-nitrosoatrazine. The total recovery of atrazine plus metabolites was 82%, whereas the total recovery of N-nitrosoatrazine and metabolites was only 39%. For atrazine, 44% of the recovered material was parent compound, whereas for N-nitrosoatrazine, only 7.4% was parent compound. A possible explanation for the low recovery of N-nitrosoatrazine and metabolites *in vivo* and *in vitro* is that N-nitrosoatrazine may be metabolized to reactive intermediates that bind to constituents of the body or the S9 faction.

No full report of the joint toxic action of atrazine and nitrate or nitrite on carcinogenic endpoints or of the carcinogenicity of N-nitrosoatrazine has been published in the peer-reviewed literature. The class of compounds to which N-nitrosoatrazine belongs, the N-nitrosamines, has been extensively studied, particularly with regard to carcinogenicity.

The N-nitrosamines have the general structure:



in which R_1 and R_2 are alkyl or aryl moieties, and may have functional groups. Depending on whether R_1 and R_2 are the same, different, or joined, the nitrosamine is called symmetrical, asymmetrical, or cyclic. N-Nitrosamines have been extensively studied. Preussmann and Stewart (1984) reported that 86% of the

232 N-nitrosamines that had been tested for carcinogenicity in animals gave positive results. Many of the remaining 14% had been tested at below the maximum tolerated dose and/or in only one species, so the apparent negative results were not definitive. A few of the tested nitrosamines were unsymmetrical alkylarylnitrosamines, as is N-nitrosoatrazine (and N-nitrososimazine) (see Appendix E for structures); some of these alkylarylnitrosamines gave positive results for carcinogenicity. In addition, "high" (relative to expected human exposures) doses of amine compounds and nitrite administered in water and/or food to rats and mice induced tumors of the same type and at the same sites as expected for the corresponding nitrosamine. Tumors did not result from the amine compound or nitrite alone. Few data are available regarding the genotoxicity of N-nitrosamines in mammalian cells, so correlations between mammalian genotoxicity and carcinogenicity cannot be established (Lijinsky 2001; Preussmann and Stewart 1984). Therefore, the potential for human cancer risk is still unresolved and further studies are needed.

2.2.5 Simazine and Nitrate

The potential for a chemical interaction between simazine and nitrite (the metabolite of nitrate) to form N-nitrososimazine has been investigated. As mentioned previously for atrazine and nitrate, the formation of N-nitrosamines from pesticide amino groups and nitrite is of concern because most N-nitrosamines are carcinogenic (Lijinsky 2001; Preussmann and Stewart 1984). Simazine and nitrite were shown to react at acidic pH to form N-nitrososimazine (Eisenbrand et al. 1975b).

An *in vivo* study also suggests the formation of N-nitrososimazine from simazine and nitrite (Dmitrenko et al. 1996). Gavage administration of radiolabled simazine at 2.3 mg/kg and sodium nitrite at 20.5 mg/kg resulted in an increase in labeled N-nitrososimazine in the liver and thymus relative to amounts formed from simazine alone at the same dose as in the mixture. Levels in kidney and spleen appeared elevated from coadministration of simazine and nitrite, but were not statistically significantly different from those obtained with simazine alone. No other tissues were analyzed. The levels of N-nitrososimazine formed in the absence of administered nitrite were attributed to endogenously formed nitrites and nitrogen oxide.

Neither simazine nor N-nitrososimazine was mutagenic in *S. typhimurium* TA98, TA100, or TA1537 with or without rat liver S9 (Ishidate 1983), but N-nitrosamines are known to be more readily activated to bacterial mutagens by hamster liver S9 than by rat liver S9 (Lijinsky 2001). Human liver S9 also may be

more active than rat liver S9 in N-nitrosamine activation, based on results with a single compound, dimethylnitrosamine (Hakura et al. 2003).

In a comparison of the potential clastogenicity of simazine and N-nitrososimazine in a Chinese hamster cell line originally established from lung fibroblasts, N-nitrososimazine produced chromosomal aberrations at 15 mg/L, but simazine did not at a 3-fold higher concentration (45 mg/L) (Ishidate 1983). This result raises the concern that the chemical interaction between simazine and nitrite may be greater than additive because it results in a new chemical that is more genotoxic than simazine. Implications for carcinogenicity or other effects are less clear.

No report of the joint toxic action of simazine and nitrate or nitrite on carcinogenic endpoints or of the carcinogenicity of N-nitrososimazine has been published. The N-nitrosamine class of chemicals, to which N-nitrosoatrazine belongs, has been studied extensively with regard to carcinogenicity. A discussion on this subject and its relevance to the potential carcinogenicity of N-nitrosoatrazine and N-nitrososimazine is provided at the end of Section 2.2.4.

2.2.6 Diazinon and Nitrate

No studies of the joint action of diazinon and nitrate were located.

2.3 Relevance of the Joint Toxic Action Data and Approaches to Public Health

This mixture was chosen because of its occurrence in rural well water. Thus, the expected exposures are intermediate to chronic oral exposures. No epidemiological or toxicological studies of the complete mixture are available. No PBPK models are available for the complete mixture or for any of the submixtures. Some information and studies are available for binary mixtures of the components, but they are not adequate to support a quantitative assessment of interactions. Therefore, the WOE approach is appropriate (ATSDR 2001a, 2001b) to predict the potential impact of interactions.

The binary weight-of-evidence (BINWOE) classification scheme is summarized in Figure 1. Rationales for the BINWOE determinations are presented in Tables 1–5 at the end of this section. The endpoints of particular interest for BINWOE determination were reproductive (atrazine, its metabolite deethylatrazine, and simazine), neurological (diazinon), hematological (nitrate), and carcinogenic (triazine reaction with nitrite to form N-nitrosamines). Insufficient information was available, however, for some of these

endpoints and binary mixtures, resulting in classifications of "indeterminate," which are not presented in the tables in this section.

The BINWOE determinations are presented for the binary mixtures in the same order as these mixtures were considered in Section 2.2. Atrazine and deethylatrazine were generally considered together as one component, because of the similarity in their metabolism and mechanism of action, and because deethyl-atrazine is a metabolite and environmental degradation product of atrazine (Appendix A).

The predicted directions of interaction were additive with high confidence for atrazine/deethylatrazine and simazine on reproductive toxicity, greater-than-additive with medium confidence for the effects of atrazine/deethylatrazine or simazine on the neurotoxicity of diazinon, and greater-than-additive with low confidence for the joint toxic action of atrazine or simazine with nitrate on carcinogenicity. BINWOEs were indeterminate for the effect of diazinon on atrazine/deethylatrazine, simazine, and nitrate, and for the effect of nitrate on diazinon.

Figure 1. Binary Weight-of-Evidence Scheme for the Assessment of Chemical Interactions*

Classification

Direction of Interaction

= Additive
> Greater than additive
< Less than additive
? Indeterminate

Quality of the Data

Mechanistic Understanding

I. Direct and Unambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has been well characterized and leads to an unambiguous interpretation of the direction of the interaction.

II. Mechanistic Data on Related Compounds: The mechanism(s) by which the interactions could occur has not been well characterized for the chemicals of concern but structure-activity relationships, either quantitative or informal, can be used to infer the likely mechanisms(s) and the direction of the interaction.

III. Inadequate or Ambiguous Mechanistic Data: The mechanism(s) by which the interactions could occur has not been well characterized or information on the mechanism(s) does not clearly indicate the direction that the interaction will have.

Toxicological Significance

A. The toxicological significance of the interaction has been directly demonstrated.

B. The toxicological significance of the interaction can be inferred or has been demonstrated for related chemicals.

C. The toxicological significance of the interaction is unclear.

Modifiers

1. Anticipated exposure duration and sequence.
2. Different exposure duration or sequence.

a. *In vivo* data
b. *In vitro* data

I. Anticipated route of exposure
ii. Different route of exposure

* Adapted from: ATSDR 2001a, 2001b

Table 1. Effect of **Atrazine/Deethylatrazine** on **Simazine**: Reproductive Toxicity
Effect of **Simazine** on **Atrazine/Deethylatrazine**: Reproductive Toxicity

BINWOE: =IA

Direction of Interaction - The direction of interaction is expected to be additive, based on a common mechanism of toxicity with regard to reproductive effects, similar metabolic fate, and additive joint toxic action on reproductive endpoints in the salmon (Moore and Lower 2001).

Mechanistic Understanding - Analysis of studies of mode of action of certain triazine herbicides, including atrazine and simazine, and their chlorinated metabolites, including deethylatrazine (metabolite of atrazine) and diaminochlorotriazine (metabolite of atrazine, deethylatrazine, and simazine), have indicated that they have a common mechanism of toxicity with regard to attenuation of the LH surge in female and male rats, alteration of the estrous cycle, delayed pubertal development in both sexes of rats, and altered pregnancy maintenance (EPA 2002c). The mechanism involves neuroendocrine disruption of hypothalamic-pituitary-gonadal function. The neuroendocrine disruption is expected to be relevant to humans. The mode of action of atrazine, deethylatrazine, and simazine with regard to these effects on reproductive function and reproductive development is expected to be dose additive. The appropriate rating for mechanistic understanding is I.

Toxicological Significance - Results of a study of the effect of atrazine and simazine on neuroendocrine and reproductive effects in mature male Atlantic salmon parr indicated concentration addition for reduced olfactory response, reduced levels of expressible milt, and on hormonal status in response to the female priming pheromone, prostaglandin $F_{2\alpha}$ (Moore and Lower 2001). Other studies of joint toxic action showed no mutagenic effects of either chemical or the mixture on *S. typhimurium* (Eisenbeis et al. 1981), and concentration addition with regard to inhibition of reproduction of cultures of *C. fusca* (green algae) (Faust et al. 1993). The toxicological relevance of the result in algae, which reflects inhibition of photosytem II, to humans is questionable. The mutagenicity study does not raise concerns for greater-than-additive toxicity. The neuroendocrine and reproductive effects in salmon have toxicological significance to the effects of concern for humans, despite the species difference. In mammals, the similarity in the reproductive effects of these chemicals together with the mechanistic understanding that indicates a common mechanism of toxicity for atrazine, deethylatrazine, and simazine strongly support the prediction of additivity. Therefore, a rating of A is chosen for toxicological significance.

Table 2. Effect of **Atrazine/Deethylatrazine** on **Diazinon**: Neurological Toxicity

BINWOE: >IIB

Direction of Interaction - The direction of interaction is expected to be greater-than-additive, based on the potentiation of diazinon neurotoxicity by atrazine in the midge (Belden and Lydy 2000), potentiation of diazinon lethality and acetylcholinesterase inhibition in amphipods (Anderson and Lydy 2002), and induction by atrazine of metabolic activation of a similar organophosphorus insecticide (Belden and Lydy 2000), and similar mechanism of neurotoxicity in invertebrates and humans.

Mechanistic Understanding - 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.

Toxicological Significance - Atrazine potentiated the acute neurotoxicity (inability of midge larvae to perform normal swimming motions) of diazinon in 96-hour static toxicity tests (Belden and Lydy 2000). Organophosphorus insecticides act as neurotoxins by inhibiting acetylcholinesterase activities in insects as well as in humans. Metabolic activation of these chemicals is similar in insects and in humans. Therefore, the result has relevance to humans. Atrazine also potentiated diazinon's inhibition of acetylcholinesterase and diazinon's acute lethality to amphipods, but did not affect diazinon's acute lethality to houseflies (Anderson and Lydy 2002). Because the mechanistic support is inferred from a atrazine's interaction with chlorpyrifos (an organophosphorus insecticide similar to diazinon), and because the potentiation was not seen in all species tested, confidence is medium and a rating of B is appropriate.

Table 3. Effect of **Simazine** on **Diazinon**: Neurological Toxicity

BINWOE: >IIB

Direction of Interaction - The direction of interaction is expected to be greater-than-additive, based on the potentiation of diazinon neurotoxicity in the midge by a similar triazine herbicide, atrazine (Belden and Lydy 2000); potentiation of diazinon lethality and acetylcholinesterase inhibition in amphipods by atrazine (Anderson and Lydy 2002); induction of metabolic activation of a similar organophosphorus insecticide, chlorpyrifos, in midges by atrazine (Belden and Lydy 2000); and on a similar mechanism of neurotoxicity in invertebrates and humans.

Mechanistic Understanding - 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. Simazine and atrazine are structurally and toxicologically similar triazine herbicides. Atrazine induced the metabolic activation of a 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, and by analogy, for simazine. Because of uncertainties inherent in extrapolating from insects to humans, and because the mechanism is inferred from similar chemicals, a rating of II is chosen for mechanistic understanding.

Toxicological Significance - Atrazine, a triazine herbicide very similar to atrazine, potentiated the acute neurotoxicity (inability of midge larvae to perform normal swimming motions) of diazinon in 96-hour static toxicity tests (Belden and Lydy 2000). Organophosphorus insecticides act as neurotoxins by inhibiting acetylcholinesterase activities in insects as well as in humans. Metabolic activation of these chemicals is similar in insects and in humans. Therefore, the result has relevance to humans. Atrazine also potentiated diazinon's inhibition of acetylcholinesterase and diazinon's acute lethality, but did not affect diazinon's acute lethality to house flies (Anderson and Lydy 2002). Because the toxicological interaction is inferred from the interaction of a related chemical (atrazine) with diazinon, the mechanistic basis is inferred from related chemicals (atrazine and chlorpyrifos), and the potentiation was not seen in all species tested, confidence is medium and a rating of B is appropriate.

Table 4. Effect of **Atrazine** on **Nitrate**: Carcinogenicity
Effect of **Nitrate** on **Atrazine**: Carcinogenicity

BINWOE: >IIC

Direction of Interaction - The direction of interaction is expected to be greater-than-additive, based on the chemical interaction of atrazine and nitrite (the metabolite of nitrate) to form N-nitrosoatrazine, a more genotoxic compound. In addition, although atrazine and nitrate/nitrite are not considered carcinogenic, N-nitrosoatrazine may possibly be carcinogenic, based on carcinogenicity data for most other N-nitrosamines (including some with similar structures) and their precursors.

Mechanistic Understanding - Atrazine and nitrite (the metabolite of nitrate) react at acidic pH to form N-nitrosoatrazine (Eisenbrand et al. 1975b; Krull et al. 1980; Mirvish et al. 1991; Wolfe et al. 1976). The formation of N-nitrosoatrazine from atrazine and nitrite has been demonstrated in soil (Kearney et al. 1977), in human gastric juice (Cova et al. 1996) and in mice (Krull et al. 1980). N-nitrosoatrazine has been tentatively identified in Mississippi River water and New Orleans drinking water (Fine et al. 1976). Thus, the evidence of N-nitrosoatrazine formation is clear. The mechanistic implications for direction of interaction are not as clear because little is known regarding the toxicity of N-nitrosoatrazine relative to its precursors, other than that it is more clastogenic and mitogenic (see *Toxicological Significance*). Nevertheless, toxicological information from most other N-nitrosamines, and from *in vivo* administration of other amine compounds with nitrite indicates that the interaction of atrazine and nitrate/nitrite to form N-nitrosoatrazine is likely to be greater-than-additive with regard to carcinogenicity, and warrants a rating of II for mechanistic understanding.

Toxicological Significance - Adequate studies of the joint toxic action of atrazine and nitrate or nitrite, or of the toxicity or carcinogenicity of N-nitrosoatrazine, were not located. Studies comparing the genotoxicity of N-nitrosoatrazine with that of atrazine or nitrate provide some relevant information. Neither atrazine nor N-nitrosoatrazine caused mutations in *S. typhimurium* with or without rat liver S9 (Ishidate 1983; Ishidate et al. 1981), but rat liver S9 is generally less effective at activating N-nitrosamines than is hamster liver S9 (Lijinsky 2001) and possibly less effective than human liver S9 (Hakura et al. 2003). The clastogenicity of N-nitrosoatrazine in cultured human lymphocytes was much greater than that of atrazine or nitrate, and N-nitrosoatrazine was mitogenic but atrazine was not (Meisner et al. 1993). N-nitrosoatrazine caused chromosomal aberrations in a Chinese hamster fibroblast-derived cell line at concentrations much lower than concentrations of atrazine that gave negative results in the same study (Ishidate 1983; Ishidate et al. 1981). Thus, N-nitrosoatrazine is more clastogenic than atrazine or nitrate, and stimulates cell division whereas atrazine does not. These results indicate that the chemical interaction of atrazine with nitrite to form N-nitrosoatrazine may be a greater-than-additive interaction for genotoxicity. Although correlations between genotoxicity in mammalian cells and carcinogenicity have not been established for the N-nitrosamines, most of the 232 N-nitrosamines that have been tested for carcinogenicity are carcinogenic, including some with structures similar to N-nitrosoatrazine (Lijinsky 2001; Preussmann and Stewart 1984). Oral administration of a variety of amine compounds together with nitrite to rats and mice has resulted in the induction of tumors of the same site and type as induced by the corresponding N-nitrosamine, whereas the parent amine compounds and nitrite were not carcinogenic (Preussmann and Stewart 1984). Structure-activity considerations raise a concern for potential carcinogenicity of this nitrosamine. However, the issue of atrazine/nitrate combination and potential cancer risk in humans is still unresolved and further studies are needed.

Table 5. Effect of **Simazine** on **Nitrate**: Carcinogenicity
Effect of **Nitrate** on **Simazine**: Carcinogenicity

BINWOE: >IIC

Direction of Interaction - The direction of interaction is expected to be greater-than-additive, based on the chemical interaction of simazine and nitrite (the metabolite of nitrate) to form N-nitrososimazine, a more genotoxic compound. In addition, although simazine and nitrate/nitrite are not considered carcinogenic, N-nitrososimazine may possibly be carcinogenic, based on carcinogenicity data for most other N-nitrosamines (including some with similar structures) and their precursors.

Mechanistic Understanding - Simazine and nitrite (the metabolite of nitrate) were shown to react at acidic pH to form N-nitrososimazine (Eisenbrand et al. 1975b). The formation of N-nitrososimazine from simazine and nitrite also has been detected in rats following oral administration (Dmitrenko et al. 1996). Thus, the evidence of N-nitrososimazine formation is clear. The mechanistic implications for direction of interaction are not as clear because little is known regarding the toxicity of N-nitrosos-imazine relative to its precursors, other than that it is more clastogenic (see *Toxicological Significance*). Nevertheless, toxicological information from most other N-nitrosamines, and from *in vivo* administration of other amine compounds with nitrite indicates that the interaction of simazine and nitrate/nitrite to form N-nitrososimazine is likely to be greater-than-additive with regard to carcinogenicity, and warrants a rating of II for mechanistic understanding.

Toxicological Significance - Adequate studies of the joint toxic action of simazine and nitrate or nitrite, or of the toxicity or carcinogenicity of N-nitrososimazine, were not located. Studies comparing the genotoxicity of N-nitrososimazine with that of simazine provide some relevant information. Neither simazine nor N-nitrososimazine caused mutations in *S. typhimurium* with or without rat liver S9 (Ishidate 1983), but rat liver S9 is generally less effective at activating nitrosamines than is hamster liver S9 (Lijinsky 2001), and possibly less effective than human liver S9 (Hakura et al. 2003). N-nitrososimazine was clastogenic at a concentration 3-fold lower than a non-clastogenic concentration of simazine (Ishidate 1983), raising the concern that a chemical interaction between simazine and nitrite may result in a new chemical that is more clastogenic than simazine. This reasoning is supported by analogy with studies of N-nitrosoatrazine, which is more clastogenic than atrazine or nitrate, and which stimulates cell division whereas atrazine does not (Ishidate 1983; Ishidate et al. 1981; Meisner et al. 1993). These results indicate that the chemical interaction of simazine with nitrite to form N-nitrososimazine may be a greater-than-additive interaction for genotoxicity. Although correlations between genotoxicity in mammalian cells and carcinogenicity have not been established for the N-nitrosamines, most of the 232 N-nitrosamines that have been tested for carcinogenicity are carcinogenic, including some with structures similar to N-nitrososimazine (Lijinsky 2001; Preussmann and Stewart 1984). Oral administration of a variety of amine compounds together with nitrite to rats and mice has resulted in the induction of tumors of the same site and type as induced by the corresponding N-nitrosamine, whereas the parent amine compounds and nitrite were not carcinogenic (Preussmann and Stewart 1984). Structure-activity considerations raise a concern for potential carcinogenicity of this nitrosamine. However, the issue of simazine/nitrate combination and potential cancer risk in humans is still unresolved and further research is needed.

2.4 Recommendations for Data Needs

Neither *in vivo* data from human or animal studies nor *in vitro* data examining the toxicity of the four-component mixture or three-component submixtures, are available. Similarly, PBPK models describing the behavior of the four-component mixture or the three- or two-component submixtures are not available. In the absence of data for the complete mixture, a component-based approach was utilized. However, mechanistic or toxicological data pertinent to the joint toxic action of diazinon and nitrate are lacking, and data for several of the pairs are not adequate to predict the direction of interaction for some toxicities, as can be readily seen from the BINWOE matrix in Chapter 3.

For the individual components, an intermediate or chronic oral MRL is available only for diazinon, but reasonably suitable health guidance values were available for the other components. A notable data gap is the lack of adequate studies of the potential carcinogenicity of N-nitrosoatrazine and N-nitroso-simazine, chemical interaction products of atrazine and simazine with nitrate.

3. Recommendation for Exposure-Based Assessment of Joint Toxic Action of the Mixture

As discussed in the introduction, the mixture of atrazine, deethylatrazine, simazine, diazinon, and nitrate was chosen as the subject for this interaction profile on the basis of an analysis of the most frequently occurring mixtures in rural domestic and public water-supply wells (Squillace et al. 2002). The exposure scenario of greatest concern for this mixture is intermediate- to chronic-duration low-level oral exposure.

No adequate epidemiological or toxicological studies and no PBPK models are available for this mixture. Recommendations for exposure-based screening for the potential health hazard of this mixture are based on ATSDR (2001a) guidance, and comprise a components-based approach. This approach is used for the components with hazard quotients that equal or exceed 0.1, when at least two of the mixture components fulfill this criterion. Hazard quotients are the ratios of exposure estimates to noncancer health guidance values, such as MRLs. If only one or if none of the mixture components has a hazard quotient of this magnitude, no further assessment of the joint toxic action is needed because additivity and/or interactions are unlikely to result in significant health hazard. As discussed by ATSDR (1992, 2001a), the exposure-based assessment of potential health hazard is a screening approach, to be used in conjunction with biomedical judgment, community-specific health outcome data, and community health concerns to assess the degree of public health hazard.

Because there are sensitive reproductive endpoints in common to the triazine components of the mixture, the recommended approach (ATSDR 2001a) for atrazine/deethylatrazine and simazine is to estimate an endpoint-specific hazard index (by summing the hazard quotients for these components) for reproductive effects, using the guidance values shown in Table 6, or newer values as they become available. Hazard quotients are the ratios of exposures to MRLs, target-organ toxicity doses (TTDs), or other health guidance values. This process is shown in the following equation:

$$HI_{REPRO} = \frac{(E_{AIR} + E_{DEA})}{TTD_{Atr\ /\ DEA\ REPRO}} + \frac{E_{Smz}}{TTD_{Smz\ REPRO}}$$

where HI_{REPRO} is the hazard index for reproductive toxicity, E_{Atr} is the exposure to atrazine (as the oral intake in mg/kg/day), E_{DEA} is the exposure to deethylatrazine (as the oral intake in mg/kg/day), and $TTD_{Atr/DEA\ REPRO}$ is the TTD (in mg/kg/day) for the reproductive effects of oral exposure to atrazine and deethylatrazine. Similarly, E_{Smz} is the exposure to simazine (as oral intake in mg/k/day) and $TTD_{Smz\ REPRO}$ is the TTD for the reproductive effects of oral exposure to simazine.

Table 6. MRLs and TTDs for Intermediate and Chronic Oral Exposure to Chemicals of Concern (See Appendices A, B, C, and D for Details)

Endpoint	Chemical			
	Atrazine[a]/ deethylatrazine (mg/kg/day)	Simazine[a] (mg/kg/day)	Diazinon (mg/kg/day)	Nitrate[a] (mg/kg/day)
Reproductive	0.003[b] 0.0018[c]	0.0018[c]	NA	NA
Neurological	NA	NA	0.0002[d]	NA
Hematological	NA	NA	NA	1.6[e]

[a]The chemical interactions of atrazine and of simazine with nitrite (a metabolite of nitrate) produce N-nitrosoatrazine and N-nitrososimazine, but adequate toxicity data do not exist to characterize the endpoints of concern or to derive health guidance values for these nitrosamines. As discussed in the text, carcinogenicity is considered a possible endpoint of concern because most other N-nitrosamines are carcinogenic.
[b]Intermediate oral MRL for atrazine
[c]Chronic dietary population adjusted dose (PAD) for atrazine and its chlorinated metabolites (combined) (EPA 2002b), adopted as target-organ toxicity dose (TTD) for atrazine and deethylatrazine (combined), and as an interim TTD for simazine.
[d]Intermediate oral MRL for diazinon.
[e]Chronic oral RfD for nitrate, adopted as TTD.

NA = not applicable

The weight-of-evidence analysis for interactions, summarized in the BINWOE determinations in Table 7, indicates that additivity is an appropriate assumption for the reproductive effects of atrazine/deethyl-atrazine and simazine, which act by a common mode of action on these endpoints, and can be considered dose additive. Confidence in the additivity assumption is high. The influence of diazinon and nitrate on the reproductive toxicity of these triazines, however, is indeterminate.

The neurological effects of diazinon are to be assessed with a separate hazard quotient for this chemical, because they are unique to the diazinon component of this mixture. This hazard quotient may underestimate the potential hazard of diazinon during co-exposure to atrazine, deethylatrazine, and simazine because the BINWOEs for the effects of these components on diazinon predict a greater-than-additive interaction (in this case, potentiation). Confidence in these predictions is medium. The influence of nitrate on the toxicity of diazinon is indeterminate.

Table 7. Matrix of BINWOE Determinations for Intermediate or Chronic Simultaneous Oral Exposure to Chemicals of Concern

		ON TOXICITY OF			
		Atrazine/ deethylatrazine	Simazine	Diazinon	Nitrate
EFFECT OF	Atrazine/ deethylatrazine		=IA r	>IIB n	? h >IIC c
	Simazine	=IA r		>IIB n	? h >IIC c
	Diazinon	? r	? r		? h
	Nitrate	? r >IIC c	? r >IIC c	? n	

r = reproductive, n = neurological, h = hematological, c = carcinogenic

The BINWOE determinations were explained in Section 2.3. No pertinent interactions data were available for the pairs of chemicals classified as indeterminate (?), and mechanistic information appeared inadequate, so indeterminate ratings were assigned to these pairs.

BINWOE scheme from ATSDR (2001a, 2001b):

DIRECTION: = additive; > greater than additive: < less than additive; ? indeterminate

MECHANISTIC UNDERSTANDING:
I: direct and unambiguous mechanistic data to support direction of interaction;
II: mechanistic data on related compounds to infer mechanism(s) and likely direction;
III: mechanistic data do not clearly indicate direction of interaction.

TOXICOLOGIC SIGNIFICANCE:
A: direct demonstration of direction of interaction with toxicologically relevant endpoint;
B: toxicologic significance of interaction is inferred or has been demonstrated for related chemicals;
C: toxicologic significance of interaction is unclear.

MODIFYING FACTORS:
1: anticipated exposure duration and sequence;
2: different exposure duration or sequence;
a: *in vivo* data;
b: *in vitro* data;
i: anticipated route of exposure;
ii: different route of exposure.

The hematological effects of nitrate also are to be assessed with a separate hazard quotient, because they are unique to the nitrate component of this mixture. The influence of the other mixture components on nitrate's hematological toxicity is indeterminate.

The potential carcinogenicity of the complete mixture is unknown. None of the individual components have been classified as carcinogenic in humans (see Appendices), but atrazine and simazine can react with nitrite, the metabolite of nitrate, to form N-nitrosoatrazine and N-nitrososimazine. The potential carcinogenicity of these nitrosamines has not been investigated adequately. Genotoxicity studies indicate they are more genotoxic than the triazines and nitrate/nitrite from which they were formed. Further exposure-based screening for cancer risk from N-nitrosoatrazine and N-nitrososimazine is not possible due to the lack of data regarding dose-response relationships for these compounds or their precursor mixtures. The potential for human cancer risk is still unresolved and further studies are needed.

If the hazard index for reproductive effects exceeds one, it provides preliminary evidence that the mixture may constitute a health hazard due to the joint toxic action of components on that endpoint (ATSDR 2001a). Similar preliminary conclusions apply if the hazard quotient for nitrate's hematological effects or diazinon's neurological effects exceeds one. The prediction that the triazines may potentiate the neurological toxicity of diazinon increases the concern, even at a diazinon hazard quotient slightly below one. If this screening procedure indicates preliminary evidence of a mixture health hazard, additional evaluation is needed to assess whether a public health hazard exists (ATSDR 2001a). This evaluation uses biomedical judgment, community-specific health outcome data, and consideration of community health concerns (ATSDR 1992).

4. Conclusions

A component-based approach is recommended for the exposure-based screening assessment of potential hazards to public health from exposure to this mixture. The recommendations include the estimation of a hazard index for the reproductive effects of the triazine components of this mixture: atrazine/deethyl-atrazine and simazine. In addition, separate hazard quotients are to be estimated for the neurological effects of diazinon and the hematological effects of nitrate. This approach is appropriate when the hazard quotients of at least two of the components equal or exceed 0.1 (ATSDR 2001a). The WOE evaluation of interactions indicates high confidence in the additivity assumption (hazard index) for atrazine/deethyl-atrazine and simazine, and uncertainty regarding the potential effect of the other mixture components on the reproductive toxicity of these triazines. Further conclusions from the WOE analysis are that the triazine components may potentiate the neurological toxicity of diazinon such that the hazard quotient may underestimate the degree of hazard; confidence in that conclusion is medium. No information regarding the impact of interactions on the hematological toxicity of nitrate was available, so uncertainty is high for this endpoint. Although the individual components of the mixture have not been classified as carcinogens, the triazine components may interact with nitrate (as the metabolite nitrite) to form N-nitrosoatrazine and N-nitrososimazine, which are more genotoxic than the parent triazine compounds. The real potential for cancer risk in humans is unresolved and further studies are needed. When the screening criteria are exceeded (hazard index above one for reproductive effects of the triazine components, hazard quotient close to or above one for neurological effects of diazinon, and/or hazard quotient above one for nitrate), further evaluation is needed (ATSDR 2001a), using biomedical judgment and community-specific health outcome data, and taking into account community health concerns (ATSDR 1992).

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Appendix A: Background Information for Atrazine and Deethylatrazine

Atrazine is a triazine herbicide (an herbicide containing the s-triazine ring) that inhibits photosynthesis in plants. Deethylatrazine is a metabolite and environmental degradation product of atrazine. The structures of these chemicals are depicted in Appendix E, and also in the metabolic scheme presented later in this appendix.

A.1 Toxicokinetics

Atrazine is rapidly absorbed from the gastrointestinal tract, based on tissue distribution in case reports of atrazine ingestion and on plasma concentrations and urinary and fecal excretion in single dose studies in rats (ATSDR 2003; EPA 2002c). Absorption of atrazine, based on excretion of atrazine and its metabolites in the urine of rats during 72–96 hours after dosing, ranged from at least 37% (one study; high dose) to at least 66% (three studies; lower doses) (EPA 2002c). Fecal excretion of atrazine and metabolites accounted for 14% of the dose in 24 hours and 19% of the dose in 72 hours after dosing (Timchalk et al. 1990). Based on the fecal excretion data, at least 81% of the dose of atrazine was absorbed.

In experimental animals and humans, atrazine is metabolized by (ATSDR 2003; EPA 2002c):

- successive N-dealkylation to deethylatrazine (desethylatrazine) or deisopropylatrazine (desisopropyl atrazine), and didealkylatrazine (commonly called diaminochlorotriazine or DACT), the major urinary metabolite;
- glutathione conjugation of atrazine and the above-listed metabolites, followed by conversion to mercapturic acid derivatives (atrazine mercapturate, deethylatrazine mercapturate, and so forth);

The dealkylation of atrazine is carried out by microsomal cytochrome P450 enzymes (ATSDR 2003; EPA 2002c). Studies with human liver microsomes indicated that CYP1A2 is the primary isozyme involved in this Phase 1 metabolism (ATSDR 2003). Studies in rat liver microsomes, conducted by a different group of investigators, initially indicated that CYP2B1 and 2C11 were the primary isozymes for atrazine metabolism in the rat (ATSDR 2003), but further *in vitro* studies by the same group concluded that CYP1A1/w is the primary isozyme involved in the dealkylation of atrazine, and that CYP 2B1/2 may be involved in hydroxylation of the isopropyl group (Hanioka et al. 1999).

Oral studies with radiolabeled atrazine in rats indicate extensive tissue distribution of radioactivity, including to the brain (EPA 2002c).

The major route of excretion is urinary (ATSDR 2003; EPA 2002c).

Studies of the toxicokinetics of deethylatrazine do not appear to be available (ATSDR 2003; EPA 2002a, 2002b, 2002c). The metabolism of deethylatrazine can be inferred from the metabolism of atrazine. Deethylatrazine is expected to be conjugated with glutathione or further dealkylated to diaminochlorotriazine, followed by conjugation with glutathione.

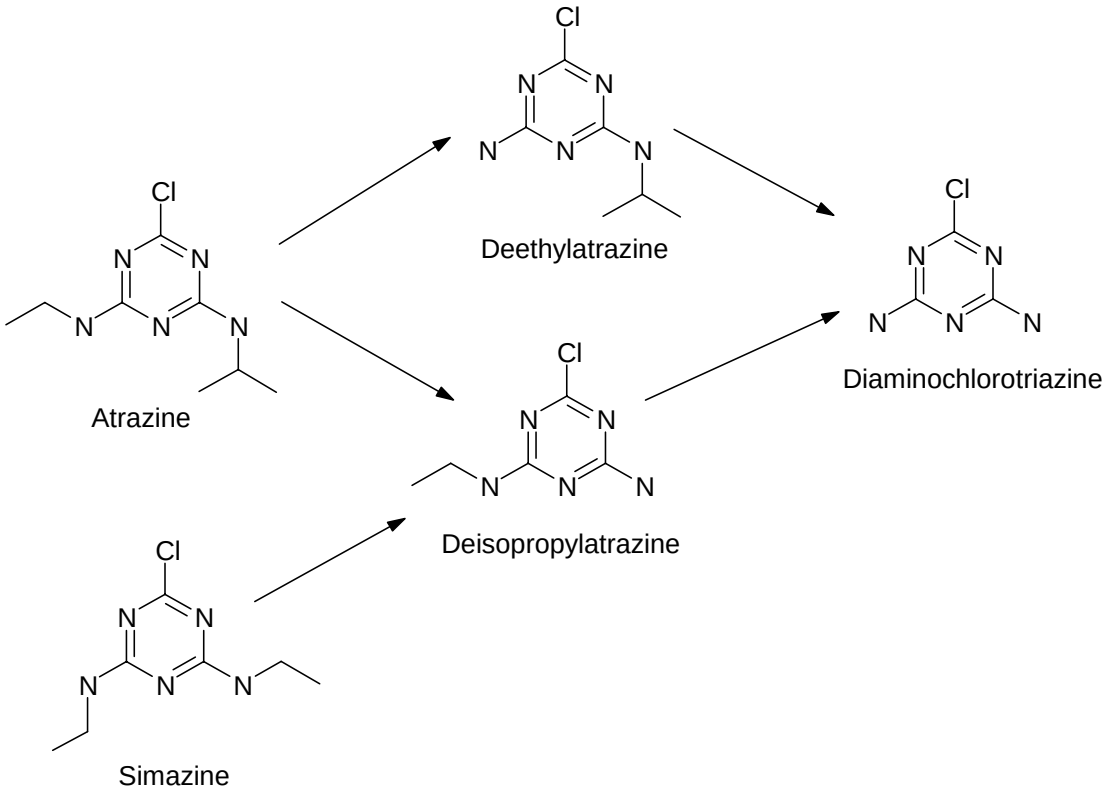
Deisopropylatrazine and diaminochlorotriazine also are metabolites of simazine. Figure 2 summarizes the metabolic dealkylation pathways that are common to atrazine and simazine, and includes deethyl-atrazine.

A.2 Health Effects

Based on results of studies in experimental animals, to be reviewed later in this section, concerns for the potential impacts of atrazine on human health include reproductive and carcinogenic effects. The epidemiological studies, however, provide little evidence of such impacts.

Three related survey studies of farm couples in Ontario investigated the potential impact of atrazine exposure (primarily direct exposure of the men) on reproductive and developmental endpoints including time to pregnancy, spontaneous abortion, preterm delivery, sex ratio, and small for gestational age (Arbuckle et al. 2001; Curtis et al. 1999; Savitz et al. 1997). These studies controlled for potential reproductive confounders, but relied entirely on self reporting of exposure and pregnancy outcome. The only significant reported association was an elevated odds ratio for preterm delivery with atrazine exposure through its use as an herbicide in the yard (but not with use on crops). Similar results were reported for triazine use in the yard (but not on crops) in the same study. The preterm delivery odds ratios were not adjusted for exposure to other pesticides (ATSDR 2003). A study of low birth weight, prematurity, and intrauterine growth retardation in Iowa communities with herbicide-contaminated municipal water supply reported an elevated relative risk for intrauterine growth retardation (adjusted for

Figure 2. Metabolic Pathways in Common to Atrazine and Simazine*



*Derived from EPA 2002c

mother's age) as compared with communities served by other water supplies (Munger et al. 1997). Results for low birth weight and prematurity were not significant. Multiple linear regression analyses revealed that, after controlling for potential confounding factors including maternal smoking, atrazine was more strongly correlated with intrauterine growth retardation than were the other herbicides, but the herbicides (atrazine, cyanazine, metolachlor) were intercorrelated. In addition, estimates of exposure and confounding factors were made on the community rather than individual level. Thus, these studies do not provide adequate evidence of reproductive effects in humans, but may indicate a need for further study.

Atrazine causes neuroendocrine, reproductive, and reproductive developmental effects in experimental animals. Animal studies have shown that atrazine disrupts estrus cyclicity (i.e., irregular ovarian cycling and changes in the number and/or percentage of days in estrus and diestrus) and alters plasma hormone levels in rats and pigs. These effects appear to be mediated by changes in the hypothalamic-pituitary-ovary axis that are species-, and even strain-, specific. In Sprague-Dawley rats, atrazine accelerates the normal process of reproductive senescence, which is initiated by a failure of the hypothalamus to release levels of gonadotropin releasing hormone (GnRH) that are adequate to stimulate the pituitary to release LH. Without sufficient LH, ovulation does not occur, estrogen levels remain high, and persistent estrus results. In other strains of rats, atrazine causes elevated progesterone levels, which leads to pseudo-pregnancy and persistent diestrus (ATSDR 2003).

The mechanism of reproductive senescence in humans does not involve disruption of hormonal regulation, but is initiated by depletion of ova in the ovaries, which ultimately results in decreased plasma estrogen levels. Therefore, disruption of the menstrual cycle or acceleration of reproductive senescence is not anticipated to occur in humans as a result of atrazine exposure. However, it is not known whether atrazine will cause other perturbations in the hypothalamus-pituitary-gonad axis resulting in reproductive effects in human (ATSDR 2003).

Developmental effects have been observed following pregestational, gestational, and lactational oral exposure of rat and rabbit dams and peripubertal oral exposure of rats to atrazine. The observed effects included impaired development of the reproductive system, postimplantation losses, decreases in fetal body weight, incomplete ossification, and neurodevelopmental effects (ATSDR 2003).

A number of epidemiology studies have investigated the carcinogenic potential of atrazine or triazine herbicides (ATSDR 2003; IARC 1999a). These studies include cohort studies of triazine manufacturing workers, case-control studies of farmers using atrazine or triazines, and ecological studies of populations

in agricultural areas with high atrazine or triazine use and populations of areas with atrazine-contaminated drinking water. Results of these studies were inconclusive. Odds ratios, standardized mortality ratios (SMRs), or relative risks generally were not elevated or were not statistically significantly elevated after adjustment for exposure to other pesticides. A few studies reported statistically significant correlations or elevated odds ratios for cancer of the prostate (Mills 1998), breast (Kettles et al. 1997), ovary (Donna et al. 1989), or stomach (Van Leeuwen et al. 1999) and triazine or atrazine exposure. These studies, however, had no individual measures of exposure and/or no accounting for exposure to other pesticides, and are not confirmed by the other available epidemiological studies on the same chemicals.

Statistically significant earlier onset or increased incidences of mammary tumors were observed in female Sprague-Dawley rats, but not in female F344 rats or in mice (ATSDR 2003). The early onset of mammary tumors in female Sprague-Dawley rats is believed to be the result of atrazine-induced acceleration of reproductive senescence, as further explained under mechanisms of action.

Deethylatrazine was not explicitly considered in the epidemiology studies. Because it is frequently detected in surface and groundwaters that contain atrazine (Gilliom et al. 1999; Squillace et al. 2002), studies that involved exposure to atrazine or triazines through drinking water probably included exposure to deethylatrazine.

A few studies of deethylatrazine have been performed in animals. In these studies, deethylatrazine generally produced the same effects as atrazine. Diaminochlorotriazine, a metabolite of both atrazine and deethylatrazine, has been tested more extensively and caused similar reproductive function and reproductive developmental effects, and carcinogenic effects (mammary gland tumors in Sprague-Dawley female rats) affects as did atrazine (EPA 2002c). Therefore, it is reasonable to assume that deethyl-atrazine will do so as well.

A.3 Mechanisms of Action

The primary target of atrazine in some animal species is the female reproductive system. Altered estrus cyclicity has been observed in Sprague-Dawley, Long-Evans, and Donryu rats following exposure to ≥5 mg/kg/day atrazine for intermediate or chronic durations and to a single dose of 300 mg/kg/day. Atrazine does not appear to have estrogenic activity. Atrazine is thought to disrupt endocrine function, and the estrus cycle, primarily through its action on the central nervous system in a manner very similar to the known mechanism of reproductive senescence in some strains of rats. In certain strains of rats,

including Sprague-Dawley and Long-Evans, reproductive senescence begins by 1 year of age, and results from inadequate stimulation of the pituitary by the hypothalamus to release LH; low serum levels of LH lead to anovulation, persistent high plasma levels of estrogen, and persistent estrus. Atrazine apparently accelerates the process of reproductive senescence in these strains of rats (ATSDR 2003).

Atrazine has been shown to induce mammary tumor formation in female Sprague-Dawley rats, but not male Sprague-Dawley or male or female F344 rats. This effect is also thought to be the result of acceleration of reproductive senescence, as described above. Both the failure to ovulate and the state of persistent estrus lead to constant elevated serum levels of endogenous estrogen, which may result in tumor formation in estrogen-sensitive tissues. The rat does not appear to be an adequate model for potential atrazine carcinogenicity in women because reproductive senescence in women involves ovarian depletion and decreased serum estrogen levels instead of decreasing hypothalamic function and increased serum estrogen levels (ATSDR 2003; EPA 2002a, 2002b, 2002c).

As previously stated, atrazine has been shown to alter serum LH and prolactin levels in Sprague-Dawley rats by altering the hypothalamic control of these hormones (Cooper et al. 2000). LH and prolactin are released from the pituitary in response to GnRH from the hypothalamus. One proposed mechanism is that atrazine decreases the hypothalamic secretion of norepinephrine, which in turn decreases the release of GnRH (EPA 2002a, 2002c). Another proposed mechanism is that atrazine disrupts hypothalamic release of GnRH by interfering with the binding of some ligands, but not others, to the $GABA_A$ receptors in a noncompetitive manner (ATSDR 2003).

A.4 Health Guidelines

ATSDR (2003) did not derive inhalation MRLs for atrazine because of the lack of suitable data.

ATSDR (2003) derived an acute oral MRL of 0.01 mg/kg/day based on a no-observed-adverse-effect level (NOAEL) of 1 mg/kg/day for decreased body weight gain in rabbits administered atrazine by gavage on gestation days 7–19, and using an uncertainty factor of 100. The LOAEL was 5 mg/kg/day; slight but statistically significant reductions in food consumption and body weight gain were seen at this dose level.

ATSDR (2003) derived an intermediate oral MRL of 0.003 mg/kg/day based on a LOAEL for delayed onset of estrus in pigs using an uncertainty factor of 300.

EPA derived an oral RfD of 0.035 mg/kg/day based on a NOAEL of 3.5 mg/kg/day in a chronic dietary study in rats, and using an uncertainty factor of 100 (IRIS 2003). The LOAEL was 25 mg/kg/day. The critical effects were decreased body weight gain in the rat study and cardiac toxicity in a 1-year dietary study in dogs. This RfD was verified by EPA in 1993; significant new studies have been published since that time (IRIS 2003).

More recently, the EPA (2002b) Office of Pesticide Programs derived an acute RfD of 0.10 mg/kg/day based on a weight-of-evidence analysis of four developmental studies, and a chronic RfD of 0.018 mg/kg/day based on attenuation of the LH surge and estrus cycle disruptions in female Sprague Dawley rats. Although not on IRIS, these derivations include a consideration of toxicological and mechanistic data that have become available since the RfD on IRIS was derived. They have been subjected to extensive review, including public comment, and are available online (EPA 2002b). A FQPA default safety factor of 10 (EPA 2003) was applied to protect infants and children (and other populations) when assessing dietary (food + drinking water) exposures, resulting in a acute population adjusted dose (PAD) of 0.01 mg/kg/day and a chronic PAD of 0.0018 mg/kg/day (EPA 2002b). These RfDs and PADs are for atrazine together with its chlorinated metabolites (including deethylatrazine), which are considered to have equivalent toxicity to atrazine.

The EPA (2002c) Office of Pesticide Programs has concluded that atrazine, deethylatrazine, diamino-chlorotriazine, deisopropylatrazine, simazine, and propazine should be considered a *Common Mechanism Group* for cumulative risk assessment due to their ability to suppress the pituitary LH surge resulting in effects on reproductive function and reproductive development.

The National Toxicology Program (NTP 2003) does not include atrazine in its listings.

The International Agency for Research on Cancer (IARC 1999a) classified atrazine as *not classifiable as to its carcinogenicity to humans* (Group 3) based on inadequate evidence in humans and sufficient evidence in experimental animals.

EPA has not published a cancer assessment of atrazine on IRIS (2003). The EPA (2002a, 2002b) Office of Pesticide Programs classified atrazine and its chlorinated metabolites (including deethylatrazine) as *not likely to be carcinogenic to humans*.

A.5 Derivation of Target Organ Toxicity Dose (TTD) Values

It is recommended that the chronic PAD of 0.0018 mg/kg/day for atrazine and its chlorinated metabolites (EPA 2002b) be adopted as a provisional TTD for reproductive effects. Details of the derivation of this guidance value are as follows: A chronic RfD of 0.018 mg/kg/day was based on an oral NOAEL for atrazine of 1.8 mg/kg/day for attenuation of the LH surge and estrus cycle disruptions in female Sprague Dawley rats (EPA 2002a, 2002b). An uncertainty factor of 100 was applied to the NOAEL (10 for interspecies extrapolation and 10 for intraspecies variations). The LOAEL for these effects was 3.65 mg/kg/day. A FQPA safety factor of 10 was applied to protect infants and children when assessing dietary (food + drinking water) exposures, resulting in chronic PAD of 0.0018 mg/kg/day. The RfD and PAD are for atrazine together with its chlorinated metabolites (including deethylatrazine), which are considered to have equivalent toxicity to atrazine (EPA 2002b).

Summary (TTD for Atrazine and Deethylatrazine)

TTD_{REPRO} = 0.0018 mg/kg/day

A.6 References

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Appendix B: Background Information for Simazine

Simazine is a triazine herbicide (an herbicide containing the s-triazine ring) that inhibits photosynthesis in plants. The structure of simazine is depicted in Appendix E, and also in the metabolic scheme presented previously in Figure 2.

B.1 Toxicokinetics

Less information is available regarding the toxicokinetics of simazine than was available for atrazine. The percent of administered radiolabel excreted in urine during 96 hours after oral dosing of rats with radiolabeled simazine was 49.3%, indicating that absorption was at least 49.3% (EPA 2002c).

In experimental animals, simazine is metabolized by (EPA 2002c; Guddewar and Dauterman 1979; IARC 1999b):

- successive N-dealkylation to deisopropylatrazine (desisopropyl atrazine), and didealkylatrazine (commonly called diaminochlorotriazine or DACT);
- glutathione conjugation of simazine and the above-listed metabolites (probably followed by conversion to mercapturic acid derivatives).

Deisopropylatrazine and diaminochlorotriazine also are metabolites of atrazine. Figure 2 summarizes the metabolic dealkylation pathways that are common to atrazine and simazine

The dealkylation of simazine is carried out by microsomal cytochrome P450 enzymes (EPA 2002c). Studies with rat liver microsomes indicate that the specific isozymes involved in this dealkylation are CYP1A1/2 (Hanioka et al. 1999).

An oral study with radiolabeled simazine in rats indicates extensive tissue distribution of radioactivity, including to the brain (EPA 2002c).

B.2 Health Effects

Some of the epidemiological studies reviewed in Appendix A were on agricultural exposure to trazines in Midwestern states, and did not specify whether exposure to simazine occurred. Because atrazine and cyanazine are the main triazines used as herbicides in the corn belt of the Midwest, it is likely that

exposures were mainly to atrazine and cyanazine (Snedeker and Clark 1998). The Ontario farm survey studies reviewed in Appendix A listed atrazine and cyanazine, but not simazine. IARC (1999b) stated that no human reproductive and developmental effects data were available for simazine, and no human cancer data were available for simazine alone.

Studies in rats indicate that simazine has effects on reproductive function and reproductive development similar to those of atrazine, as do its metabolites deisopropylatrazine and diaminochlorotriazine (EPA 2002c). Also, simazine and diaminochlorotriazine cause mammary gland tumors in Sprague-Dawley female rats (EPA 2002c). As explained previously for atrazine, this carcinogenic effect of simazine is not considered relevant to humans (see Section A.2 and A.3).

B.3 Mechanisms of Action

The mechanism of action of simazine and its metabolites deisopropylatrazine and diaminochlorotriazine is considered to be the same as for atrazine as described in Section A.3 with regard to neuroendocrine, reproductive, and carcinogenic effects (EPA 2002c).

B.4 Health Guidelines

ATSDR has not developed a toxicological profile or MRLs for simazine.

EPA derived an oral RfD of 0.005 mg/kg/day based on a NOAEL of 0.52 mg/kg/day in a chronic dietary study in rats, and using an uncertainty factor of 100 (IRIS 2003). The LOAEL was 5.3 mg/kg/day. The critical effects were reduction in weight gain and hematological changes (mainly depression of red cell parameters. This RfD was verified by EPA in 1993; significant new studies have been published since that time (IRIS 2003).

More recently than the 1993 assessment that is on IRIS (2003), the EPA (2002c) Office of Pesticide Programs concluded that atrazine, deethylatrazine, diaminochlorotriazine, deisopropylatrazine, simazine, and propazine should be considered a *Common Mechanism Group* for cumulative risk assessment due to their ability to suppress the pituitary LH surge resulting in effects on reproductive function and reproductive development. These effects were considered the critical effects. Taking into account newer toxicological and mechanistic data, EPA (2002b) developed new RfDs for atrazine and its chlorinated metabolites (see Section A.4), but has not yet developed new RfDs for simazine.

NTP (2003) does not include simazine in its listings.

IARC (1999b) classified simazine as *not classifiable as to its carcinogenicity to humans* (Group 3) based on inadequate evidence in humans and sufficient evidence in experimental animals.

EPA has not published a cancer assessment of simazine on IRIS (2003).

B.5 Derivation of Target Organ Toxicity Dose (TTD) Values

It is recommended that the chronic PAD of 0.0018 mg/kg/day for atrazine and its chlorinated metabolites (EPA 2002b) also be adopted as a provisional TTD for reproductive effects for simazine. The derivation of this guidance value is described in Section A.5. The structure, molecular weights, metabolism, toxicity, and mechanisms of action of these chemicals are similar, and they are considered to belong to a *Common Mechanism Group* for cumulative risk assessment due to their ability to suppress the pituitary LH surge resulting in effects on reproductive function and reproductive development (EPA 2002c). This value is recommended as an interim measure until an up-to-date guidance value is developed specifically for simazine.

Summary (TTD for Simazine)

TTD_{REPRO} = 0.0018 mg/kg/day

B.6 References

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Appendix C: Background Information for Diazinon

Diazinon is an organophosphorus insecticide. The structure of diazinon and its toxic metabolite, diazoxon, are provided in Appendix E.

C.1 Toxicokinetics

Diazinon is rapidly absorbed from the gastrointestinal tract, based on case reports of ingestion of diazinon formulation or solution, on single oral dose studies in rats and dogs, and on repeated oral dose studies in rats. Absorption in rats and dogs was at least 85% of the dose (ATSDR 1996; WHO 1998). The main features of diazinon metabolism are:

- activation of diazinon through conversion of the P=S moiety to P=O, resulting in the toxic intermediate, diazoxon;
- cleavage of the ester bonds of diazinon and diazoxon resulting in 2-isopropyl-4-methyl-6-hydro-pyrimidine (from both), diethylphosphorothioc acid (from diazinon), and diethylphosphoric acid (from diazoxon);
- oxidation of the isopropyl substituent of 2-isopropyl-4-methyl-6-hydropyrimidine to the corresponding primary and tertiary alcohols;
- glutathione-mediated cleavage of the ester bond with the formation of a glutathione conjugate (minor pathway).

The resulting metabolites are excreted primarily in the urine (ATSDR 1996; WHO 1998).

The metabolic activation of diazinon to diazoxon is carried out by microsomal cytochrome P450 monooxygenases. A single study of diazinon in rat hepatic microsomes has reported that CYP2B1/2 are the major P450 isozymes that catalyze the production of diazoxon (Fabrizi et al. 1999).

C.2 Health Effects

The principal toxic effect of diazinon in humans, experimental animals, and insects is acetylcholinesterase inhibition. Acetylcholine is a neurotransmitter in the central and peripheral neurons. Inhibition of acetyl-cholinesterase, the enzyme that breaks down and terminates the action of acetylcholine, results in the accumulation of acetylcholine at acetylcholine receptors leading to continued stimulation.

In humans and experimental animals, the accumulation of acetylcholine results in cholinergic responses in the peripheral (muscarinic and nicotinic) and central nervous system and neuromuscular junctions. These cholinergic responses, seen in severe acetylcholinesterase inhibition, include excessive glandular secretions (salivation, lacrimation, rhinitis), miosis, bronchoconstriction, vasodilation, hypotension, diarrhea, nausea, vomiting, urinary incontinence, and bradycardia associated with muscarinic receptor stimulation. Tachycardia, mydriasis (dilation of the pupil), muscle fasciculations, cramping, twitching, muscle weakness, muscle paralysis, and hypertension are associated with nicotinic receptor stimulation. Central nervous system toxicity includes respiratory depression, anxiety, insomnia, headache, apathy, drowsiness, dizziness, loss of concentration, confusion, tremors, convulsions, and coma. These effects usually appear within a few minutes to 24 hours after exposure, depending on the extent and route of exposure. In nonfatal exposures, the effects are usually transient, with rapid and complete recovery following cessation of exposure. Recovery from diazinon poisoning results from increased availability of active acetylcholinesterase either from synthesis of new enzyme, the spontaneous hydrolysis of the enzyme-phosphate ester complex, or treatment with atropine, a competitive antagonist of acetylcholine at muscarinic and central nervous system receptors, and with pralidoxime (2-PAM), a drug that regenerates inhibited acetylcholinesterase enzyme by displacing the diethylphosphoester bond that diazoxon forms at the active site (Aaron and Howland 1998; ATSDR 1996).

In some cases, however, diazinon may cause a condition known as the intermediate syndrome (Aaron and Howland 1998; WHO 1998). This syndrome occurs during apparent recovery about 24–96 hours after severe cholinergic crisis, and includes paralysis of the respiratory muscles, upper extremity muscles, neck flexors, and motor cranial nerves. Diazinon has been tested for organophosphate-induced delayed neurotoxicity in chickens; results were negative (ATSDR 1996). No cases of delayed neuropathy from diazinon exposure have been reported (ATSDR 1996; WHO 1998).

Acetylcholinesterase activity is also present in erythrocytes where it is known as erythrocyte acetyl-cholinesterase. Both forms of acetylcholinesterase are produced by the same gene and are kinetically identical. In *in vitro* assays, erythrocyte and neural acetylcholinesterase are inhibited to roughly the same extent by exposure to diazinon and many other organophosphorus compounds with insecticidal activity; measurement of erythrocyte acetylcholinesterase can be used as a surrogate indicator of the extent of inhibition of neural acetylcholinesterase (ATSDR 1996).

A cholinesterase capable of hydrolyzing acetylcholine and butyrlcholine is produced by the liver and circulates in the blood. This enzyme, referred to as serum cholinesterase, plasma cholinesterase, pseudo-

cholinesterase, or butyrylcholinesterase, is also inhibited by diazinon and is often used as a marker for exposure (ATSDR 1996). This enzyme is present in some nonneural cells in the central and peripheral nervous systems as well as in plasma and serum, the liver, and other organs. Its physiologic function is not known, but is hypothesized to be the hydrolysis of esters ingested from plants (Lefkowitz et al. 1996). Plasma cholinesterases are also inhibited by organophosphate compounds through irreversible binding; this binding can act as a detoxification mechanism as it affords some protection to acetylcholinesterase in the nervous system (Parkinson 1996; Taylor 1996). In general, this enzyme is inhibited by diazinon at lower levels of exposure than required to inhibit neural or erythrocyte acetylcholinesterase (ATSDR 1996).

A few case reports of diazinon ingestion or dermal exposure have reported acute pancreatitis as a component of severe diazinon intoxication (ATSDR 1996; WHO 1998). Diazinon at sublethal doses also caused pancreatic ductal hypertension in dogs, and acute pancreatitis in dogs and guinea pigs but not in cats (Dressel et al. 1980; Frick et al. 1987). Effects on the pancreas appear to be a high-dose phenomenon.

Epidemiological studies provide no specific evidence of carcinogenicity for diazinon, and the available animal studies do not suggest that diazinon would be likely to cause cancer in humans (ATSDR 1996; EPA 2000).

C.3 Mechanisms of Action

Diazinon and diazoxon inhibit acetylcholinesterase by reacting with the active site to form a stable dialkylphosphorylated enzyme that cannot hydrolyze acetylcholine. Diazoxon, the active metabolic intermediate of diazinon, is much more potent than diazinon in inhibiting acetylcholinesterase (ATSDR 1996; WHO 1998).

The mechanism of action with regard to pancreatic toxicity in dogs and guinea pigs appears to be inhibition of butyrylcholinesterase in the pancreas and its smooth muscle sphincters, leading to ductal hypertension and cholinergic hyperstimulation of the acinar cells (Dressel et al. 1980; Frick et al. 1987).

C.4 Health Guidelines

ATSDR (1996) derived an intermediate inhalation MRL of 0.009 mg/m^3 for brain acetylcholinesterase inhibition diazinon based on a NOAEL of 0.46 mg/m^3 in a 21-day study in rats. An uncertainty factor of 30 was applied. The LOAEL (20% decrease in brain acetylcholinesterase) was 1.57 mg/m^3.

ATSDR (1996) derived an intermediate oral MRL of 0.0002 mg/kg/day based on a NOAEL of 0.021 mg/kg/day for brain acetylcholinesterase inhibition in dogs given diazinon in their food daily for 13 weeks. An uncertainty factor of 100 was used. The LOAEL (31% decrease in erythrocyte and brain acetylcholinesterase) was 5.9 mg/kg/day. A chronic oral MRL was not derived because the chronic NOAEL (0.05 mg/kg/day for brain cholinesterase inhibition in rats) that was considered as the basis for the MRL would have resulted in an MRL (0.0005 mg/kg/day) that was slightly higher than the intermediate oral MRL. Since the intermediate-duration MRL would be more protective, it was the only one derived, and was considered protective for individuals living near hazardous waste sites.

EPA (IRIS 2003) does not have an online file for diazinon.

The EPA (2000) Office of Pesticide Programs derived acute and chronic RfDs of 0.0025 and 0.0002 mg/kg/day based on NOAELs for cholinesterase inhibition of 2.5 mg/kg/day (in rats) and 0.02 mg/kg/day in seven feeding studies (in rats and dogs), respectively. An additional 10-fold FQPA safety factor (EPA 2003) was not used for special sensitivity in infants and children because the EPA concluded that the data indicated that this factor could be reduced to 1-fold. The PADs are therefore the same as the RfDs. Although this RfD and PAD are not on IRIS, they have been subjected to extensive review, including public comment, and are available online (EPA 2000).

NTP (2003) and IARC (2003) do not include diazinon in their listings. The EPA (2000) Office of Pesticide Programs classified diazinon as a *not likely human carcinogen* based on the lack of evidence of carcinogenicity in mice and rats.

C.5 Derivation of Target-Organ Toxicity Dose (TTD) Values

The intermediate oral MRL of 0.0002 mg/kg/day for neurological effects (brain acetylcholinesterase inhibition in dogs for 13-week oral exposure) is appropriate for use as a chronic guidance value as well (ATSDR 1996), and is the same as the chronic oral RfD developed by EPA (2000). An uncertainty factor

of 100 was applied to a NOAEL of 0.021 mg/kg/day, as described in the previous section. The LOAEL (31% decrease in erythrocyte and brain acetylcholinesterase) was 5.9 mg/kg/day.

Summary (TTD for Diazinon)

MRL_{NEURO} =0.0002 mg/kg/day

C.6 References

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Appendix D: Background Information for Nitrate

Nitrate occurs naturally in foods, particularly in vegetables. Inorganic fertilizers, livestock waste, and septic tank discharges are primary contributors to nitrate contamination of drinking water (NRC 1995). The structures of nitrate and its metabolite nitrite are shown in Appendix E.

D.1 Toxicokinetics

Available studies indicate that oral absorption of nitrate is nearly 100% (for reviews, see EPA 1990 and WHO 1978). Witter (1979, cited in EPA 1990) administered oral radioactive nitrate ion to two male volunteers; one received the nitrate 1 hour after a large meal, the other about 10 hours after eating. In the subject who had recently eaten, the radioactivity had a disappearance half-life from the stomach of about 30 minutes, but the radioactivity in the pylorus remained constant, suggesting that the nitrate had moved to the small intestine rather than being absorbed through the stomach. In the second subject, the disappearance half-life was 10 minutes. Studies in animals have also demonstrated that the bulk of an orally-administered nitrate is absorbed through the small intestine, likely through the upper portion of that organ. Absorbed nitrate is distributed throughout the body, but does not appear to accumulate in any organ (EPA 1990).

The major metabolic pathway for nitrate is conversion to nitrite, and then to ammonia. Small amounts of nitrate, perhaps 5–10% of the total exposure, are converted to nitrite by bacteria in the saliva, stomach, and small intestine. This reaction is pH dependent, with no nitrate reduction occurring below pH 4 and above pH 9, and the presence of oxygen inhibits the reduction of nitrite to ammonia. Absorbed nitrite rapidly reacts with hemoglobin in the blood to form methemoglobin, which in adults, is rapidly converted to oxyhemoglobin, then back to hemoglobin. In infants, particularly those under 3 months old, these reducing systems are not fully developed, which may result in a buildup of methemoglobin in the blood. Due to the higher stomach pH typically found in infants, it is believed that they also convert more nitrate to nitrite in the stomach than adults. There are large species differences in the rate of reaction of nitrite with hemoglobin, paralleled by similar differences in the rates of reduction of methemoglobin, making extrapolation of results from animal data to humans problematic. Another potential metabolic pathway, though less prevalent than the reaction with hemoglobin, is the reaction of nitrite with endogenous molecules to form N-nitroso compounds, many of which have toxic effects, including carcinogenicity.

Available data in humans have demonstrated that excretion of ingested nitrate is rapid, with excretion almost exclusively in the urine (EPA 1990; WHO 1978). Animal data support this observation. In both

humans and animals, considerably more nitrate is eliminated in the urine than is ingested in a normal diet, implying that there is significant endogenous nitrate formation.

Parks et al. (1981, cited in EPA 1990) reported that following intratracheal instillation of trace amounts of nitrate to BALB/C mice, absorption from the lungs was complete within a 10-minute period. Additional studies of the toxicokinetics of inhaled nitrate are not available; however, the behavior of absorbed nitrate following inhalation exposure is not expected to differ from nitrate absorbed following oral exposure.

D.2 Health Effects

The most sensitive known effects of exposure to nitrate result from increased levels of methemoglobin arising from the nitrite-hemoglobin reaction. In healthy adults, methemoglobin formation and reduction is continuous, with steady-state methemoglobin levels in healthy adults being 2.5% of the total hemoglobin content or lower (EPA 1990). Due to the large excess capacity of the blood to carry oxygen, levels of methemoglobin up to 10% typically do not cause significant clinical signs. Levels above 10% may result in cyanosis, weakness, rapid pulse, and, at levels exceeding 50%, death. Other reported effects of nitrate in animals include altered thyroid function, amyloidosis of the liver, kidney, spleen, and adrenal glands, and altered lung and liver weights.

Because of greater numbers of nitrate-reducing bacteria in the gastrointestinal tract and diminished methemoglobin-reducing capacity, infants, especially those 3 months and younger, are particularly susceptible to nitrate/nitrite-induced methemoglobinemia. A study by Bosch et al. (1950) examined 139 cases of methemoglobinemia in young children (90% of these cases occurred in children <2 months of age). Examination of the wells used to supply water to the children revealed that none of the wells supplied <10 mg/L nitrate-nitrogen, with all but two of the wells containing >25 mg/L. Walton (1951) presented the results of a survey on morbidity and mortality among infants due to methemoglobinemia. The results of the survey revealed 239 cases of infant methemoglobinemia, 39 of them fatal. Of the 214 cases where quantitative data were available on nitrate levels in water, none occurred in infants consuming water with <10 mg/L nitrate-nitrogen, 5 cases occurred in infants exposed to 11–20 mg/L nitrate-nitrogen, 36 cases in infants exposed to 21–50 mg/L nitrate-nitrogen, and 173 cases in infants exposed to >50 mg/L nitrate-nitrogen. Many other studies have examined the effects of high (>20 mg/L) levels of nitrate in the drinking water of infants, and have found increased methemoglobin levels and signs of clinical methemoglobinemia in exposed infants (for reviews, see EPA 1990 and WHO 1978).

The Nuclear Regulatory Commission (NRC 1995), in its evaluation of the drinking water maximum contaminant level goals (MCLGs) and MCLs for nitrate and nitrite, discussed the possible contributions of infection and inflammatory reactions (particularly diarrhea in infants) to methemoglobinemia. Infection increases the production of nitric oxide, which can be converted to nitrate. Avery (1999) reviewed the evidence for gastrointestinal infection and inflammation as a cause of methemoglobinemia in infants. Most of the studies of nitrate and infant methemoglobinemia are not adequate to clarify this issue. A recent nested case-control study of methemoglobinemia risk factors, however, reported a stronger association of infant methemoglobinemia with nitrate exposure (from formula and tea made with nitrate-contaminated water) than with diarrhea (Zeman et al. 2002).

The nitrite ion and various organic nitrate compounds (e.g., nitroglycerin) cause vasodilation and hypotension, but inorganic nitrate ion does not (EPA 1990).

D.3 Mechanisms of Action

The known toxic effects of nitrate exposure result from the conversion of nitrate to nitrite. The conversion is mainly the result of bacterial oxidation reactions within the gastrointestinal tract. Exposure of hemoglobin to nitrite results in the oxidation of the Fe^{2+} ion in the heme of hemoglobin to Fe^{3+}, resulting in the formation of methemoglobin. Methemoglobinemia results in the majority of the symptoms seen following high-dose acute nitrate exposure in humans. Under normal conditions, healthy adults will have <2.5% methemoglobin in the blood. Methemoglobin can be reduced back to hemoglobin by both spontaneous (nicotinamide adenine dinucleotide phosphate [NADH]-dependent) and dormant (NADPH-dependent) methemoglobin reductase enzymes.

Infants are particularly susceptible to methemoglobinemia due to their high gut content of nitrate-reducing bacteria, their lower enzymatic capacity to reduce methemoglobin to hemoglobin, and the presence of hemoglobin F, which is more susceptible to oxidation by nitrite. The high pH of the infant gastrointestinal system favors the growth of nitrate-reducing bacteria, particularly in the stomach and especially after ingestion of contaminated waters, since the ingested bacteria are likely to flourish in the stomach. The stomach of adults is typically too acidic to allow for significant bacterial growth and the resulting conversion of nitrate to nitrite. Additionally, the enzymes involved in the conversion of methemoglobin to hemoglobin do not fully develop in humans until between 3 and 6 months after birth, resulting in an increased susceptibility to methemoglobinemia.

As mentioned in Section D.1, the reaction rates for the nitrite-hemoglobin reaction vary considerably across species (many animal species lack nitrate-reducing bacteria), as do the rates of the reactions reducing methemoglobin back to functional hemoglobin. In addition, since the rates of conversion of nitrate to nitrite by bacteria can vary within individuals, the extent of nitrate toxicity can vary greatly depending on age and other factors within both humans and animals.

D.4 Health Guidelines

ATSDR has not published a toxicological profile for nitrates. No MRL values are available.

EPA (IRIS 2003) has derived an oral RfD of 1.6 mg/kg/day for nitrate, based on a NOAEL of 1.6 mg/kg/day for methemoglobinemia in exposed infants (Bosch et al. 1950; Walton 1951). An uncertainty factor of 1 was applied to the NOAEL since the study was performed in a sensitive population of humans (infants age 0–3 months).

NTP (1993) and IARC (2003) do not include nitrate in their listings. Nitrate has not undergone an evaluation of carcinogenic potential by EPA (IRIS 2003).

D.5 Derivation of Target Organ Toxicity Dose (TTD) Values

In the absence of a toxicological profile and MRLs for nitrate, the chronic oral RfD of 1.6 mg/kg/day for nitrate (IRIS 2003) can be adopted as the TTD for hematological effects.

Summary (TTD for Nitrate)

TTD_{HEMATO} = 1.6 mg/kg/day

D.6 References

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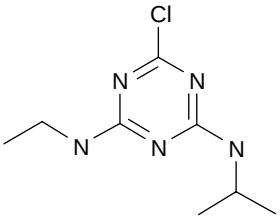
Appendix E: Chemical Structures of Organic Mixture Components



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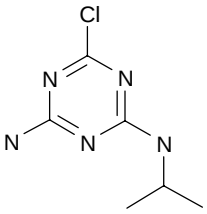

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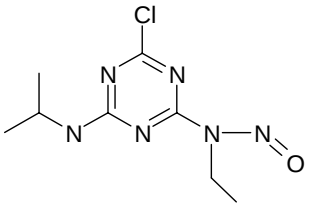

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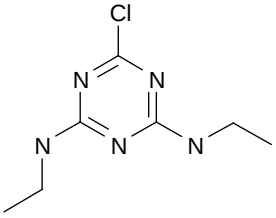

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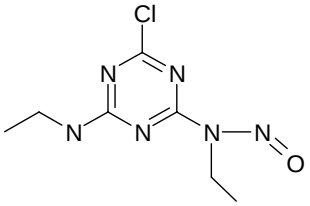

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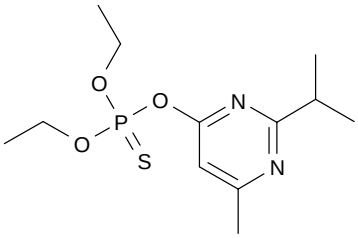

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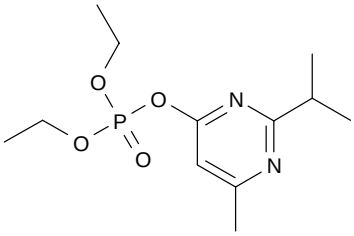

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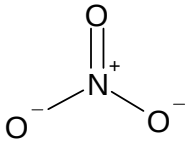

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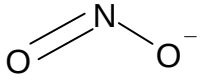

Nitrite
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