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.

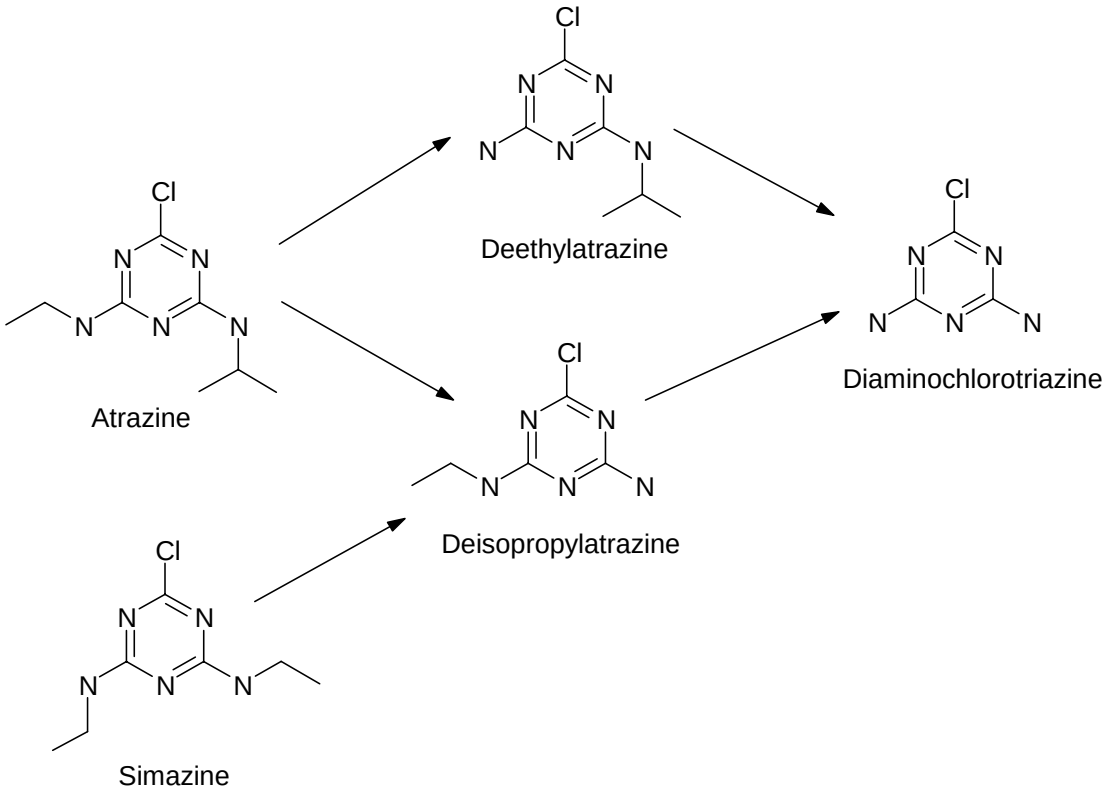
Deisopropylatrazine and diaminochlorotriazine also are metabolites of simazine. Figure 2 summarizes the metabolic dealkylation pathways that are common to atrazine and simazine, and includes deethyl-atrazine.

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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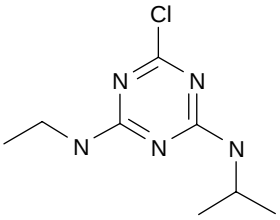
Appendix E: Chemical Structures of Organic Mixture Components



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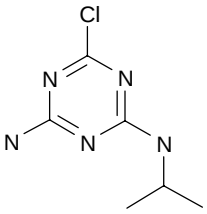

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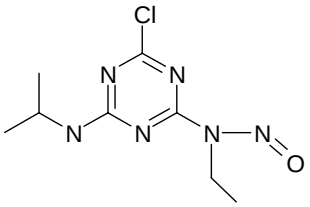

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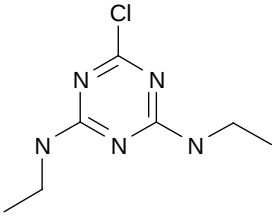

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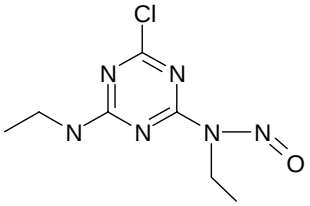

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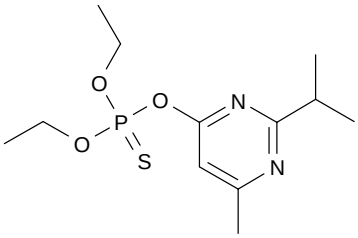

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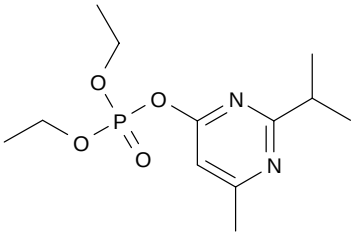

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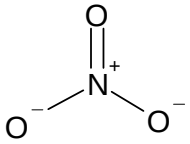

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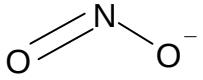

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