

PRIORITY DATA NEEDS FOR GUTHION

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NOTE TO THE READER

The Priority Data Needs documents are intended to characterize substance-specific priority data needs determined via the ATSDR Decision guide for identifying substance-specific data needs related to toxicological profiles (54 Federal Register 37618, September 11, 1989). The identified priority data needs reflect the opinion of the Agency, in consultation with other federal programs, of the research necessary for fulfilling its statutory mandate under the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (Superfund) or CERCLA. They are not intended to represent the priority data needs for any other program.

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Substance-Specific Applied Research Program

Priority Data Needs for:

Guthion

Prepared by: Agency for Toxic Substances and Disease Registry/
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I. Executive Summary

Guthion is included in the priority list of hazardous substances identified by ATSDR (ATSDR 2007). This list contains substances that have been identified at National Priorities List (NPL) sites and determined to pose a human health risk based on (1) known or suspected human toxicity, (2) frequency of occurrence at NPL sites or other facilities, and (3) the potential for human exposure to the substance. The Toxicological Profile for Guthion was published by ATSDR in September 2008.

Guthion (also called azinphos-methyl) is an organophosphate insecticide that was used on many crops, especially apples, pears, cherries, peaches, almonds, and cotton. All of guthion's remaining uses are scheduled to be cancelled by the year 2010. It is estimated that the current production and use of guthion in the United States is <2 million pounds annually. Pure guthion is a colorless to white, odorless, crystalline solid with a melting point range of 72–74 °C, while the technical-grade material is a cream to yellow-brown, granular solid with a melting point of 67–70 °C. Guthion is readily soluble in most organic solvents (acetone, toluene, chloroform, acetonitrile, benzene, xylene, carbon tetrachloride, and chlorobenzene), slightly soluble in methanol, ethanol, and propanol, and poorly soluble in water. Volatilization of guthion from soil and water surfaces is not considered an important environmental fate process.

Guthion is not highly persistent in the environment, and degrades by a combination of biotic and abiotic mechanisms. Biodegradation occurs readily in soils and water under aerobic conditions with half-lives on the order of several days to a few weeks. Hydrolysis and photolysis are also important degradation pathways for guthion in water, foliage, and soils. In the atmosphere, vapor-phase guthion is quickly degraded by photochemically produced hydroxyl radicals; the

half-life for this reaction in air is on the order of a few hours. Particulate-phase guthion is removed from the atmosphere by wet and dry deposition processes. Guthion has moderate to low mobility in soils. Its leaching potential is considered low, and therefore guthion is only occasionally detected in groundwater.

The most important route of exposure to guthion for the general population, including children, is through the ingestion of foods, especially vegetables and fruits that have been sprayed with this insecticide. Ingestion of contaminated drinking water, inhalation exposure, and dermal exposure to guthion are expected to be low for the general population. Agricultural workers, their families, and persons residing near crops that are treated with guthion are expected to have much greater frequency of exposure and the potential to be exposed to higher levels of guthion than the general population. There are insufficient data to determine whether populations residing near hazardous waste sites will be exposed to higher levels of guthion than the general population, and the primary route of exposure for these persons is likely to be similar to that of the general population (e.g. ingestion of contaminated food).

The toxicity of guthion has been studied in animals exposed via inhalation, oral, or dermal routes. The observed reductions in erythrocyte or brain cholinesterase activity as well as clinical signs of neurotoxicity indicate that the nervous system is the critical target of toxicity for guthion. Systemic effects were generally observed at doses that were higher than those associated with reductions in cholinesterase activity or clinical signs of neurotoxicity. Significant reductions in brain weight and brain cholinesterase activity were observed in the only study that examined the developmental toxicity of guthion in pups from mothers exposed to guthion from mating through lactation. A cancer bioassay in rats and mice provided equivocal evidence of the carcinogenic potential of guthion in male rats. The cancer bioassay also suggested that guthion exposure may elicit endocrine effects, as shown by an increase in the incidence of cystic endometrial hyperplasia in female mice; however, it cannot be ascertained at this time whether or not guthion is an endocrine disruptor.

On the basis of the available data, ATSDR has identified the following priority data needs:

Exposure

- No exposure priority data needs have been identified.

Toxicity

- Studies of developmental toxicity via oral exposure with emphasis on neurodevelopmental toxicity.

II. Introduction: ATSDR's Substance-Specific Applied Research Program

A. Legislative

Section 104(i)(5) of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of guthion is available. Where adequate information is not available, ATSDR, in cooperation with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine these health effects. Such program shall include, to the extent necessary to supplement existing information, but shall not be limited to--

- laboratory and other studies to determine short, intermediate, and long-term health effects;
- laboratory and other studies to determine organ-specific, site-specific, and system-specific acute and chronic toxicity;
- laboratory and other studies to determine the manner in which such substances are metabolized or to otherwise develop an understanding of the biokinetics of such substances; and
- where there is a possibility of obtaining human data, the collection of such information.

Section 104(i)(5)(C): In the development and implementation of the research program ATSDR is required to coordinate with EPA and NTP to avoid duplication of research being conducted in other programs and under other authorities.

Section 104(i)(5)(D): It is the sense of Congress that the costs for conducting this research program be borne by private industry, either under the Toxic Substances Control Act (TSCA), the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), or cost recovery under CERCLA.

B. Impact on Public Health

The major purpose of this research program is to supplement the substance-specific informational needs of the public and the scientific community. More specifically for ATSDR, this program will supply necessary information to improve the database to conduct public health assessments. This is more fully described in the ATSDR Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (54 Federal Register 37618) [henceforth referred to as the ATSDR Decision Guide].

Experience from ATSDR health assessments shows the need for more information for select substances, on both exposure and toxicity, so the Agency can more completely assess human health effects. Exposure data collected from this substance-specific research will complement data being collected on a site-specific basis by ATSDR's Division of Health Studies and the Division of Health Assessment and Consultation. More specifically, the Agency will use the exposure data to help identify populations that need follow-up exposure or health-outcome studies.

Regarding substance toxicity, the collected data will be used to characterize the toxicity of the substance for public and scientific community. For ATSDR, the data are necessary and essential to improve the design and conduct of follow-up health studies.

C. Procedures

Section 104(i)(2) of CERCLA, as amended, requires that ATSDR (1) with EPA develop a list of hazardous substances found at NPL sites (in order of priority), (2) prepare toxicological profiles of those substances, and (3) assure the initiation of a research program to fill identified data needs associated with the substances.

The first step in implementing the ATSDR substance-specific research program for guthion occurred when the data needs for guthion were determined in the ATSDR Toxicological Profile for guthion. Considered a subset of all information gaps on guthion, these data needs were reviewed by scientists from ATSDR and other federal agencies. They were peer reviewed by an external review panel and made available for public comment. All comments received by

ATSDR on the identification of data needs for guthion were addressed before the toxicological profile was finalized.

The purpose of this paper is to take the data needs identified in the Toxicological Profile for guthion and subject them to further scientific evaluation. This will lead to priorities and ultimately to ATSDR's substance-specific research agenda. To affect this step, ATSDR developed and presented a logical scientific approach to priority setting in its Decision Guide.

Briefly, data needs are categorized as exposure or toxicity and are then subcategorized across three levels (Tables 1 and 2). Level I research is a base set of exposure and toxicity information to identify basic characteristics of each substance. Level II research is conducted to confirm the toxicity and exposure indicated by Level I data. Level III research will improve the application of the results of Level II research to people.

The Decision Guide recognized three general principles for setting priorities:

- Not all information gaps identified in toxicological profiles are data needs.
- All data needs are not the same priority.
- Substances should be considered individually, but may be grouped, because of structural similarity or other relevant factors.

Other considerations spelled out in the Decision Guide include:

- All levels of data should be considered in selecting priority data needs.
- Level I gaps are not automatically in the priority grouping. In general, Level I data have priority when there are no higher level data for the same category, and when data are insufficient to make higher level priority testing decisions. For example, priority would generally not be assigned multigenerational animal studies (Level II) if an adequate subchronic study (Level I) had not been conducted that evaluated reproductive organ histopathology.
- Priority for either exposure or toxicity data requires thorough evaluation of research needs in other areas to help achieve a balanced research program for each substance.

The Decision Guide listed the following eight tenets to determine research priorities:

- Development and/or confirmation of appropriate analytical methods.

- Determination of environmental and human exposure levels when analytical methods are available.
- Bioavailability studies for substances of known significant toxicity and exposure.
- Studies available to characterize target organs and dose response.
- Disposition studies and comparative physiologically-based pharmacokinetics when a toxic end point has been determined and differences in species response have been noted.
- Mechanistic studies on substances with significant toxicity and substantial human exposure.
- Investigation of methods to mitigate toxicity for substances when enough is known about mode of action to guide research.
- Epidemiologic studies designed to link human disease with a substance of known significant toxicity.

These last three "prioritizing" tenets address Level III research. When Level III research is identified as priority, ATSDR will not develop detailed methods to successfully fulfill the data needs. Because there are no standard "testing guidelines" for Level III research, we expect considerable discussion between ATSDR and parties interested in conducting this research. Thus, ATSDR will only announce that its scientists believe that the accumulation of Level III research is appropriate, and it is a priority at this time. ATSDR will state the reasons why this is so.

D. Selection Criteria

ATSDR prepares toxicological profiles on substances that are most commonly found at facilities on the NPL sites and which, in its sole discretion, pose the most significant threat to human health because of their known or suspected toxicity and potential for human exposure.

Briefly, the rationale is as follows:

1. Frequency of Occurrence

Finding: Guthion is included in the priority list of hazardous substances identified by ATSDR (ATSDR 2007).

Guthion has been detected in at least 5 of 1,678 National Priorities List (NPL) hazardous waste sites in the United States (HazDat 2006). Exposure to guthion at these sites may occur by contacting contaminated air, water, soil, or sediment. ATSDR is presently evaluating the extent of media-specific contamination at these and other sites.

2. Potential for Human Exposure

Finding: ATSDR scientists have determined that there has been significant past human exposure and that the potential exists for current human exposure to guthion via inhalation, ingestion, and skin contact.

The following is a brief summary of the potential for human exposure to guthion. For a more detailed discussion of available information, refer to the ATSDR Toxicological Profile for guthion, Chapter 6, on Potential for Human Exposure (ATSDR 2008).

Guthion is a nonvolatile colorless to white odorless crystalline solid or cream to yellow-brown granular solid that is soluble in most organic solvents such as acetone, toluene, chloroform, acetonitrile, benzene, xylene, carbon tetrachloride, and chlorobenzene, but poorly soluble in water. Guthion is a broad spectrum organophosphate insecticide, acaricide, and molluscicide that has been used to control a wide variety of insects including codling moths, plum curculios, apple maggots, aphids, leafrollers, mites, mealybugs, moths, and boll weevils (EPA 2001). It has been used on a variety of crops; however, its major use has been on tree crops, including pome and stone fruit and nut crops (EPA 2001). On June 9, 2006 EPA proposed the cancellation of all remaining uses of guthion. This includes guthion's use for apples, blueberries, cherries, parsley, and pears by 2010 and cancellation of its uses on almonds, Brussels sprouts, pistachios, walnuts, and nursery stock by 2007 (EPA 2006).

Guthion is an important substance for research because of its widespread environmental contamination. No information is available in the TRI database on facilities that manufacture or process guthion because this chemical is not required to be reported under Section 313 of the Emergency Planning and Community Right-to-Know Act (Title III of the Superfund Amendments and Reauthorization Act of 1986) (EPA 1997). Guthion is primarily released to the environment as a result of its use as an insecticide. In 1997, 2,091,014 pounds of guthion were used on crops throughout the United States with the vast majority being applied to apple orchards

(USDA 2000). This represented an 18% decrease from national usage data compiled for 1992 in which 2,548,867 pounds were used. The EPA estimated that the annual use of guthion is currently <2 million pounds.

Guthion is typically applied to foliage of treated crops through ground spray equipment, although aerial applications using light weight aircraft also occur. Guthion sprayed to crops eventually settles to soil, although it may be released to water from spray drift, runoff, and erosion of treated soils. If released to soil or water, volatilization is not expected to be an important environmental fate process based on a Henry's law constant of 3.7×10^{-9} atm-m³/mol at 25 °C (EPA 1999a) and vapor pressure of 2.2×10^{-7} mm Hg (Suntio 1988). Adsorption/desorption experiments using three different soils suggest that guthion has moderate to low mobility in soil and the potential to leach into groundwater is considered low. The K_{oc} values of guthion in a sandy loam (1.6% organic carbon), silt loam (2.9% organic carbon), and clay loam (0.3% organic carbon) were calculated as 475, 579, and 3,266, respectively (EPA 1999a). Guthion is not persistent in the environment and degrades by a combination of biotic and abiotic mechanisms. The time for 50% dissipation (DT_{50}) of guthion applied to a sandy loam soil and incubated under aerobic conditions was 27 days (EPA 1999a). The DT_{50} of guthion in laboratory studies employing four different soils from Italy ranged from 4 to 20 days (Diaz Diaz 1995). The shortest dissipation times were observed in alkaline soils that were high in organic matter. Field dissipation studies using alfalfa fields in California indicated a fairly rapid rate of dissipation. Guthion applied at a rate of 3 pounds a.i./A in August to a Salinas silt loam (pH 6.9–8.0) located in Watsonville, California had a DT_{50} of 9 days (EPA 1999a). A similar experiment was conducted using an alfalfa field in Fresno, California during the month of May. The soil type in this field was characterized as a Hesperia fine sandy loam (pH 7.6–8.7). The DT_{50} was 2 days in this soil following a single application at 3 pounds a.i./A (EPA 1999a). The hydrolysis half-lives of guthion at 30 °C in aqueous buffered solutions at pH 4, 7, and 9 were 49, 26, and 3.7 days, respectively (EPA 1999a). The aqueous photolysis half-life of guthion maintained at pH 4.35 and 30 °C and exposed to natural sunlight conditions in Kansas City, Missouri was calculated as 76.7 hours (EPA 1999a). Guthion applied directly to foliage appears to degrade very rapidly under field conditions. The presence of sensitizing agents in leaves and vegetation can result in enhanced photolysis, thus increasing the degradation rates of pesticides in sunlight (Floesser-Mueller and Schwack 2001). Foliar degradation half-lives of guthion on plants and leaves have been reported to range from 1.6 to 16.0 days (EPA 1999a).

Guthion has been identified in at least 5 of the 1,678 hazardous waste sites that have been proposed for inclusion on the EPA NPL (HazDat 2006). However, the number of sites evaluated for guthion is not known. Guthion was detected in air and groundwater samples at one site each, and soil samples at three NPL sites where guthion was detected in some environmental media.

The general population, including children, is primarily exposed to guthion through the ingestion of fruits and vegetables that have been treated with this insecticide. Residue monitoring data from the U.S. Department of Agriculture's Pesticide Data Program (USDA-PDP) supplemented with information from the Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition (CFSAN) were collected on approximately 44 different food commodities between the years 1994 and 2000. Guthion was detected in 3,897 out of 54,047 samples collected. In general, guthion was detected at levels below 1 ppm in most food items, although a single maximum occurrence of 1.9 ppm was reported for guthion in pears (EPA 2002). The dietary average daily intake (AVDI) of guthion for eight different age and gender groups was estimated from market basket surveys conducted by the FDA from 1986 to 1991. The dietary AVDI of guthion ranges from about 4 to 31 ng/kg/day (Gunderson 1995). Ingestion of contaminated drinking water, inhalation exposure, and dermal exposure to guthion are expected to be low for the general population.

Agricultural workers, their families, and persons residing near crops that are treated with guthion are expected to have much greater frequency of exposure and the potential to be exposed to higher levels of guthion than the general population. Data regarding exposures of residents living near hazardous waste sites could not be located.

3. Toxicity

Finding: ATSDR considers that short, intermediate, and long-term health effects can result from inhalation, ingestion, and dermal contact of guthion. Target organs or systems known to be affected include the nervous system, specifically, neural and erythrocyte acetylcholinesterase.

The following is a brief summary of the toxicology of guthion. Refer to the ATSDR Toxicological Profile for guthion, Chapter 3, on "Health Effects" for a more detailed discussion of available information (ATSDR 2008).

The available human and animal data suggest that reductions in cholinesterase activity are the most sensitive end points of the toxicity of guthion. In both humans and animals, erythrocyte acetylcholinesterase inhibition occurs at doses that are several times lower than those that elicit clinical signs and symptoms. The neurotoxicity of guthion is dependent on its bioactivation via a cytochrome P450 mediated desulfuration to the oxon form (Buratti et al. 2003), known as the azinphosmethyl oxon (Sultatos and Woods 1988) or gutoxon (Hitchcock and Murphy 1971). Gutoxon inhibits the enzymatic action of nervous system cholinesterase on the neurotransmitter acetylcholine, leading to the accumulation of acetylcholine at the ending of cholinergic nerves with the ensuing continual stimulation of electrical activity (Carrier and Brunet 1999).

There is a paucity of data regarding the inhalation, oral, and dermal toxicity of guthion in humans. Limited data are available in studies of the effect of guthion on human erythrocyte and plasma cholinesterase activity. These studies reported no significant changes in plasma or erythrocyte cholinesterase activity in a small group of subjects ingesting guthion daily for 4 weeks (Rider and Puletti 1969; Rider et al. 1970, 1971, 1972). An increased association has been suggested between the occurrence of systemic illnesses (defined as an acute illness following pesticide exposure, with symptoms and signs not restricted to the eyes or skin) in workers and agricultural use of guthion (Weinbaum et al. 1997). Although studies of agricultural workers have used the detection of urinary metabolites of guthion (Franklin et al. 1981; Schneider et al. 1994) and cholinesterase activity monitoring (Kraus et al. 1977; Schneider et al. 1994) to demonstrate exposure to guthion, no symptoms or signs of organophosphate poisoning were observed in the exposed workers even with documented reductions of 10–20% in erythrocyte (Schneider et al. 1994) or whole blood (Kraus et al. 1977) cholinesterase activity. These findings are in agreement with animal studies, which indicate that erythrocyte cholinesterase activity is very sensitive to guthion and that clinical signs in laboratory animals exposed to guthion are generally observed at concentrations that are several times higher than those that elicit reductions in erythrocyte cholinesterase activity. Studies with rats and dogs suggest that reductions in erythrocyte cholinesterase activity are not related to exposure duration (Allen 1990; Astroff and Young 1998; Holzum 1990; Schmidt and Chevalier 1984; Sheets et al. 1997). Erythrocyte cholinesterase activity is more sensitive than plasma or brain cholinesterase activity to the toxic effects of guthion. Biologically significant reductions in erythrocyte cholinesterase activity were observed in male and female rats exposed to guthion via inhalation for up to 12 weeks, but brain cholinesterase activity was not affected and plasma cholinesterase activity was reduced only in females at one sampling time (Kimmerle et al. 1976). Reductions in brain and plasma

cholinesterase activity in rats and dogs were generally observed at doses that were approximately twice the dose that elicited reductions in erythrocyte cholinesterase activity (Allen 1990; Astroff and Young 1998; Holzum 1990; Pasquet et al. 1976; Schmidt and Chevalier 1984; Sheets et al. 1997).

No association was detected between occupational exposure to guthion and the occurrence of congenital malformations in a study of male agricultural workers (García et al. 1998). Single oral doses in mice during gestation elicited reductions in fetal body weight and skeletal anomalies (Kavlock et al. 1985). Adverse developmental outcomes such as skeletal abnormalities, decreased pup weight and survival, reduced brain weight and cholinesterase activity, and neuromuscular effects were observed in the offspring of pregnant rats or mice treated with guthion during gestation (Short et al. 1980) and gestation and lactation (Holzum 1990). The adverse developmental outcomes observed in the study by Short et al. (1980) occurred at levels associated with maternal mortality. Developmental effects were not evident in rats or mice at oral doses ≤ 2.5 mg/kg/day (Astroff and Young 1998; Short et al. 1980). Reductions in litter and pup viability were observed in the fetuses of pregnant mice after a single oral dose of 20 mg/kg (Kavlock et al. 1985) and in the offspring of rats after exposure to 1.3 mg/kg/day during gestation and lactation (Holzum 1990).

Guthion does not appear to be an immunotoxicant. Guthion was not a dermal sensitizer or an irritant (Lisi et al. 1987; Sartorelli et al. 1999). Vos et al. (1983) reported reduced spleen and mesenteric lymph node weights in rats administered guthion at 11.5 mg/kg/day, but not at 2.3 mg/kg/day, for 3 weeks.

No studies were located that have examined the carcinogenic potential of guthion in humans. A carcinogenicity assay in rats and mice administered guthion in the diet for 80 weeks is available (NCI 1978). Under the conditions of the bioassay, NCI (1978) concluded that guthion was not carcinogenic in male or female mice or female rats. The incidences of neoplasms of the pancreatic islets and of the follicular cells of the thyroid in male rats provide equivocal evidence of the carcinogenic potential of guthion in male rats. Significant increases, relative to pooled controls, were observed in the combined incidence of islet cell adenoma or carcinomas of the pancreas in male rats and benign thyroid tumors, malignant thyroid tumors, or combined follicular cell tumors in male rats (NCI 1978); however, these tumors cannot be clearly implicated to a chemically induced effect because the observed incidences in male rats in this

study fall within the range of the spontaneous incidence of these lesions observed in male rats in the conducting laboratory (NCI 1978). There was no evidence of the occurrence of treatment-related tumors in a study of male and female Wistar rats exposed to 0.25–3.11 mg/kg/day for 2 years (Schmidt and Chevalier 1984). The Department of Health and Human Services (NTP 2005) and IARC (2006) have not classified guthion as to its carcinogenicity. In 1993, EPA concluded that there was a lack of evidence of carcinogenicity of guthion in male and female mice and rats (EPA 1999b, 2001b). EPA (IRIS 2006) currently has no carcinogenicity classification for guthion.

III. Identification of Data Needs

In evaluating the exposure and toxicity testing needs for guthion, ATSDR considered all available published and unpublished information that has been peer-reviewed. From its evaluation of these data, ATSDR is recommending the conduct of specific research or testing.

A. Exposure Data Needs (Table 1)

Three of the eight "prioritizing" tenets presented in the Decision Guide directly address exposure data needs:

- Development and/or confirmation of appropriate analytical method;
- Determination of environmental and human exposure levels when analytical methods are available; and
- Bioavailability studies for substances of known significant toxicity and exposure.

The progressive accumulation of exposure information begins with developing suitable analytical methods to analyze the compound in all relevant biological and environmental media, followed by confirmation of exposure information, before the conduct of any Level III research. However, in order to know what analytes are available to monitor, some basic environmental fate information is generally required and becomes a priority if it is lacking.

Bioavailability and food chain bioaccumulation studies are appropriately placed in Level II, and should be undertaken after analytical methods are developed and the substance has been confirmed at many hazardous waste sites and in environmental media.

1. Levels I & II Data Needs

a. Analytical Methods

Purpose: To determine if available methods are adequate to detect and quantify levels of guthion in environmental and biological matrices. The methods should be sufficiently specific and sensitive to measure (1) background levels in the environment and the population; and (2) levels at which biological effects might occur.

Finding: A data need has been identified. There are insufficient methods available that can detect guthion levels in biological fluids. It is difficult to monitor for exposure to guthion in humans because the biological half-life of guthion ranges from approximately 24 to 36 hours in humans (California EPA 2004; Loewenherz et al. 1997). Although an analytical method has been developed for determining the level of guthion in blood and urine (Pitarch et al. 2001), it is primarily applicable in cases of acute guthion ingestion or poisoning. Exposure to guthion is usually analyzed by measuring the level of urinary metabolites dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), and dimethyl dithiophosphate (DMDTP) in the urine (Koch et al. 2002) or measuring cholinesterase activity in plasma, red blood cells, and whole blood (Vasilic et al. 1987). Measuring the cholinesterase activity and these three metabolites are not specific to guthion, however, and may be present due to exposure to other organophosphates.

Methods for determining guthion levels in air (Foreman et al. 2000; NIOSH 1994), water (EPA 1998; 2000), soil (EPA 2000a; Gamon et al. 2003), sediment (Knuth et al. 2000; Villa et al. 2003), and various foods (Danis et al. 2002; Kyriakidis et al. 2001; Sheridan and Meola 1999) exist. These methods are sufficiently sensitive to measure levels in the environment that approach ATSDR's Environmental Media Evaluation Guides (EMEGs) calculated from ATSDR's Minimal Risk Levels (MRLs) and background levels and levels at which biological effects might occur. No additional analytical methods for determining low levels of guthion in environmental media are needed at this time.

Priority Recommendation: Although a data need exists for the development of analytical methods specific to guthion, it is not considered priority at this time because it may not be feasible to measure a chemical with a short biological half-life such as guthion. However,

analytical methods are available for measuring the metabolites of guthion even though they are not specific to guthion, but may arise due to exposure from several organophosphates.

b. Physical/Chemical Properties

Purpose: To determine whether adequate data on the chemical and physical properties of guthion are available to permit estimation of its environmental fate under various conditions of release, and evaluation of its pharmacokinetics under different exposure durations and routes.

Finding: A data need has not been identified. The physical and chemical properties of guthion are sufficiently well defined to allow assessments of the environmental fate of this compound to be made. The most important properties such as Henry's law constant (EPA 1999a), vapor pressure (Suntio et al. 1988), solubility (Tomlin 2003), log K_{ow} (Hansch et al. 1995), melting point (Tomlin 2003), and boiling point (EPA 2001) have been measured.

Priority Recommendation: A data need has not been identified.

c. Exposure Levels

(1) Environmental Media

Purpose: To determine whether adequate data are available on the levels of guthion in the ambient and contaminated environments for purposes of conducting meaningful follow-up exposure and health studies.

Finding: A need to obtain reliable and current data on concentrations of guthion in contaminated environmental media at hazardous waste sites has been identified.

Levels of guthion are generally low in areas where it has not been applied as an insecticide. Weekly composite rainfall samples that were obtained in urban and agricultural regions of the Midwestern United States and along the Mississippi River indicated a low frequency of detection for guthion from April to September 1995 (Majewski et al. 2000). Guthion was not detected in any samples of rainfall from a background location (Eagle Harbor, Michigan) where it had no known use. Guthion was detected in approximately 10% of the rainfall samples collected in

agricultural areas of Mississippi and in approximately 5% of the rainfall samples collected in an urban area (Jackson, Mississippi). Guthion was not detected in rainfall samples obtained in either agricultural or urban areas of Iowa, but was detected in approximately 1% of the rainfall samples collected in an agricultural location in Minnesota (Majewski et al. 2000). During the same collection period, guthion was identified, not quantified, in approximately 20% of the vapor-phase and particulate-phase air samples collected from Rolling Forks, Mississippi (agricultural location), but was not detected in air samples collected in Jackson, Mississippi (Coupe et al. 2000; Foreman 2000). Guthion was detected in 36% of the atmospheric samples obtained near locations in Kern and Glenn Counties, CA where it was being used as an insecticide on almond crops (Baker et al. 1996). The 24-hour mean concentration was $0.035 \mu\text{g}/\text{m}^3$ and the maximum concentration was $0.11 \mu\text{g}/\text{m}^3$. The maximum concentration observed in the air at the application site was $1.6 \mu\text{g}/\text{m}^3$ (Baker et al. 1996).

Guthion was only detected (detection limit $0.001 \mu\text{g}/\text{L}$) in 4 out of 2,451 groundwater samples collected from 1992 to 1996 in 20 major hydrological basins across the United States (Kolpin et al. 2000). The maximum observed concentration in these four positive samples was $0.18 \mu\text{g}/\text{L}$. Guthion was not detected in 94 shallow groundwater wells sampled in 1992 in the Midwestern United States (Kolpin et al. 1995). Very little data exist for guthion in finished drinking water; however, limited monitoring data suggest that its occurrence is not widespread. In a cumulative risk assessment for organophosphate pesticides, the EPA Office of Pesticide Programs (OPP) performed a 2-year pilot reservoir monitoring study of raw and finished water data for 18 active organophosphate parent compounds and 13 transformation products (EPA 2002). Guthion was detected in 8 out of 321 raw water samples at a mean concentration of $0.077 \mu\text{g}/\text{L}$ and a maximum concentration of $0.144 \mu\text{g}/\text{L}$. Guthion was detected in 5 out of 225 finished drinking water samples at a mean concentration of $0.059 \mu\text{g}/\text{L}$ and a maximum concentration of $0.114 \mu\text{g}/\text{L}$. Due to spray drift, runoff, and erosion of treated soils, guthion is frequently detected in surface waters adjacent to farming areas where it has been applied as an insecticide. Guthion was detected in 64 out of 98 surface water samples at a maximum concentration of $0.523 \mu\text{g}/\text{L}$ obtained from various sites in a heavy apple growing region along the Yakima River Basin, Washington during the period of May 1999 through January 2000 (USGS 2001).

Soil samples collected from 48 homes of agricultural families in eastern Washington State had mean guthion levels of $60 \mu\text{g}/\text{kg}$ (range: not detected to $814 \mu\text{g}/\text{kg}$), while soil samples collected from 11 homes of nonagricultural families had no detectable levels of guthion (detection limit

32 µg/kg) (Simcox et al. 1995). For the homes of the agricultural families, a positive correlation was observed between guthion levels in the soil and household dust, and the proximity to nearby apple orchards (Simcox et al. 1995). In a study of 49 randomly chosen agrichemical facilities located throughout the state of Illinois, guthion was detected in soil samples at 5 of the 10 sites that processed, used, or handled it (Krapac et al. 1995). The mean, median, and range of guthion concentrations in the soil samples at these five sites were reported as 148, 110, and 45–878 µg/kg, respectively.

Priority Recommendation: The identified need is not considered priority. Reliable and current monitoring data for the levels of guthion in contaminated media at hazardous waste sites are needed so that the information obtained on levels of guthion in the environment and the resulting body burden of guthion can be used to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites. However, ATSDR has developed a hazardous substance release/health effects database (HazDat) that includes the extant data for the 5 NPL sites at which guthion has been found. This database includes maximum concentrations of guthion in on- and off-site media, and an indication of relevant routes of exposure. Further evaluation of this database is needed first to assess if collection of additional media-specific data is assigned priority.

(2) Humans

Purpose: To determine whether adequate data are available on the levels of guthion in human tissues for the general population and exposed populations for purposes of conducting meaningful follow-up exposure and health studies.

Finding: A need has been identified. No data are available on the levels of guthion in body tissues or fluids for people living near hazardous waste sites.

There are no data regarding guthion levels in various human tissues and body fluids for the general population. Urinary metabolites that are reflective of exposure to guthion were measured as a part of the National Health and Nutrition Examination Surveys (NHANES) (CDC 2005). The geometric means (95% confidence interval) of DMP, DMTP, and DMDTP urinary level for all ages were 13.4, 32.6, and 4.95 µg/L, respectively, for 2001–2002 monitoring (CDC 2005). These dialkyl phosphate metabolites are not specific to guthion, but their detection indicates the

possibility of exposure to guthion and several other organophosphate pesticides. Dialkyl phosphates may also be present in the environment from the degradation of these pesticides. Therefore, in addition to reflecting exposure to guthion or other organophosphate pesticides, the presence of the metabolites in a person's urine may also reflect exposure to the metabolite itself (CDC 2005).

Priority Recommendation: The identified data need to collect additional information is not considered priority because analytical methods are not currently available that can readily determine guthion levels in biological fluids.

d. Exposures of Children

Purpose: To determine if adequate data on exposures of children to guthion are available for the purpose of conducting meaningful follow-up exposure and health studies.

Finding: A data need has been identified. There are no exposure studies or body burden measurements of guthion in children. Similar to adults, children are primarily exposed to guthion through the ingestion of foods. The average dietary intake of guthion has been reported as 0.069–0.083 µg/kg-body weight/day for 6–11-month-old infants and 0.022–0.031 µg/kg-body weight/day for 2-year-old toddlers (Gunderson 1988, 1995). No measurements have been made of guthion in amniotic fluid, meconium, cord blood, neonatal blood, or any other tissues that may indicate prenatal exposure. No data have been reported on the levels of guthion in breast milk. The metabolite DMP was detected in 1 out of 20 postpartum meconium samples obtained from newborn infants at the New York Presbyterian Hospital (Whyatt and Barr 2001). The metabolites DMTP and DMDTP were not detected.

Nondietary ingestion may be an important exposure pathway in agricultural areas, where guthion is used as an insecticide. The exposure of young children to organophosphate pesticides, including guthion, in an agricultural community in central Washington was studied by collecting spot urine and hand wipe samples from a group of 109 children aged 6 months to 6 years during the pesticide spraying months of May–July (Lu et al. 2000). Participants included 62 agricultural families (49 applicators and 13 farm workers) and 14 reference families in which no family member was employed in occupations requiring contact with pesticides, and the residence was located at least one quarter mile away from any pesticide treated orchard. There were 72, 19, and

18 children of pesticide applicators, farm workers, and reference families, respectively. The median urinary levels of the dimethyl metabolites DMTP and DMDTP were 0.05 µg/mL in the children of the agricultural families and 0.01 µg/mL in the children of reference families (Lu et al. 2000). Approximately 67% of the urine samples collected from the children of pesticide applicators and farm workers contained detectable levels of DMTP, while 53% of the urine samples collected from the children of reference families contained detectable levels. Wipes obtained from the hands of the children indicated that detectable levels of guthion were present in approximately 13% of the children's hands from agricultural families, while none of the children from the reference families had detectable levels of guthion in hand wipe samples. Additional exposure to guthion may also arise from the clothing or personal items of adults who are employed in pesticide application or other farm work. The mean guthion level on the surface of work boots in the agricultural families was 0.03 µg/cm² and the mean level on the steering wheel of the family vehicle was 0.001 µg/cm² (Lu et al. 2000). Guthion was not detected on personal clothing items or in the vehicles of the 14 reference families.

Priority Recommendation: The identified data need to conduct additional studies to assess exposures of children to guthion is not considered priority. Collecting information on the levels of guthion in children is important in order to determine the extent of a child's exposure to guthion as well as to identify ways to reduce the potential sources for exposure risks. However, due to the rapid biological half-life of guthion, analytical methods are not currently available that can readily determine guthion levels in biological fluids. Analytical methods are available for the urinary metabolites of guthion, dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), and dimethyl dithiophosphate (DMDTP); however, these metabolites are not specific to guthion and may result from exposure to several other organophosphate pesticides.

e. Environmental Fate

Purpose: To determine whether the available data are adequate to estimate exposure to guthion under various conditions of environmental release for purposes of planning and conducting meaningful follow-up exposure and health studies.

Finding: A data need has not been identified. Sufficient data are available to characterize the environmental fate of guthion. When applied as an insecticide, guthion adsorbs strongly to soil surfaces and is degraded in the environment by a combination of biotic and abiotic reactions. It

may enter nearby water bodies through spray drift, runoff, and erosion of treated soils where it is expected to partition to suspended solids and sediment. The aerobic biodegradation half-life of guthion in various soils ranges from a few days to approximately a month (Diaz Diaz 1995; EPA 1999a). The hydrolysis half-life of guthion at 30 °C in aqueous buffered solutions at environmental pH (pH 4–9) ranged from approximately 4 to 49 days (EPA 1999a). Guthion was shown to undergo photolysis in aqueous solutions exposed to natural sunlight with a photolysis half-life of approximately 3 days (EPA 1999a).

Based on K_{oc} values in the range of 475–3,266 in three different soils in the United States (EPA 1999a) and K_{oc} values in the range of 534–4,644 in five standard European soils (Gawlik et al. 1998), guthion is expected to possess moderate to low mobility in soil and the potential to leach into groundwater is considered low. Based upon a Henry's law constant of 3.7×10^{-9} atm-m³/mol at 25 °C (EPA 1999a) and vapor pressure of 2.2×10^{-7} mm Hg (Suntio 1988), volatilization from soil and water surfaces is not expected to be an important environmental fate process.

A minor amount of guthion that may partition to air during its spray application is expected to be degraded rapidly. Vapor-phase guthion is degraded in the atmosphere through reaction with photochemically produced hydroxyl radicals and direct photolysis. An estimated hydroxyl radical rate constant of 1.5×10^{-10} cm³/molec-sec was estimated for guthion using a structure-estimation method (Meylan and Howard 1993). This corresponds to an atmospheric half-life of approximately 2.5 hours, assuming an atmospheric hydroxyl radical concentration of 5×10^5 molec/cm³ (Atkinson 1985). In a direct photolysis study, thin films of guthion exposed to summer sunlight at Riverside, California degraded with an approximate half-life of 8.2 hours (Chukwudebe et al. 1989).

Priority Recommendation: A data need has not been identified.

f. Bioavailability and Bioaccumulation Potential

Purpose: To determine whether adequate data are available to predict the potential of guthion to be taken up by people exposed via contaminated air, soil, water, and the food chain, in order to plan and conduct meaningful follow-up exposure and health studies.

Finding: A data need has been identified. Guthion is absorbed following both oral and dermal exposures (Fakhr et al. 1996; Franklin et al. 1986). Guthion primarily partitions to soil following its application to crops as an insecticide. No experimental studies were located regarding the bioavailability of guthion from contaminated soil; therefore, data are needed regarding the bioavailability of guthion from this environmental medium.

There are little data regarding guthion's potential to bioconcentrate in aquatic organisms. An estimated bioconcentration factor (BCF) of 26 was calculated from a log K_{ow} of 2.75 (Hansch et al. 1995) and a regression-derived equation (Meylan et al. 1999). This BCF value suggests that the potential for guthion to bioconcentrate and bioaccumulate in aquatic organisms is low. However, experimental studies using constructed ecosystems indicate that guthion may bioconcentrate in aquatic organisms. Guthion formulated as an emulsifiable concentrate and applied to the surface of a 2 ha pond near Duluth, Minnesota at a nominal application rate of 20 µg/L showed accumulation in fathead minnows (Knuth et al. 2000). A maximum lipid corrected BCF value of 3,003 was observed 3 hours postapplication, while a minimum value of 1,027 was observed 1 day postapplication. Eight days postapplication, the BCF gradually increased to 2,254 (Knuth et al. 2000). Although these data indicate a high degree of bioconcentration, the whole-body BCF values in the minnows are substantially lower. Using the author-reported mean lipid content of 2.12% in the fathead minnows, the maximum whole-body BCF value is approximately 64 (3 hours postapplication), and the minimum value is approximately 22. These whole-body BCF values indicate that bioconcentration in aquatic organisms is low to moderate. These data are consistent with the findings of uptake and accumulation studies conducted using catfish. Catfish exposed to guthion had a relatively low magnitude of accumulation with rapid uptake and excretion (California EPA 2004).

Priority Recommendation: The identified data need is not considered priority since the uptake and absorption of guthion from soils is not considered the primary route of exposure for the general population (including children) and persons living near hazardous waste sites.

2. Level III Data Needs

a. Registries of Exposed Persons

Purpose: To help assess long-term health consequences of exposure to guthion in the environment. The ATSDR Division of Health Studies will be asked to consider this substance for selection as a primary contaminant to establish a guthion subregistry of the National Exposure Registry.

Finding: A data need has been identified. Guthion has been found in at least 5 NPL hazardous waste sites. At this time, no formal registries exist that identify people known to have been exposed to guthion. The development of an exposure registry should provide an important reference tool to help assess long-term health consequences of exposure to guthion. It should also facilitate the conduct of epidemiologic or health studies to assess any increased incidence of chronic disease or late-developing effects such as cancer. An effort is currently under way at ATSDR to identify those sites where humans have been exposed to site contaminants. From those identified sites, ATSDR can determine which sites list guthion as a contaminant and the size of the potentially exposed population.

Priority Recommendation: The identified data need is not considered priority. The development of a guthion subregistry at this time would not contribute significantly to the current database. The development of an exposure subregistry should await the results of needed studies on analytical methods to monitor guthion in biological matrices as well as information on levels in populations living near hazardous waste sites.

B. Toxicity Data Needs (Table 2)

The five remaining "prioritizing" tenets presented in the Decision Guide address toxicity data needs.

- Studies available for all toxicological profile substances to characterize target organs and dose response.
- Disposition studies and comparative physiologically-based pharmacokinetics when a toxic end point has been determined and differences in species response have been noted.
- Mechanistic studies on substances with significant toxicity and substantial human exposure.
- Investigation of methods for mitigation of toxicity for substances where enough is known about mode of action to guide research.

- Epidemiologic studies that will provide a direct answer on human disease for a substance of known significant toxicity.

The following is a brief summary of the toxicity data needs for Guthion. Please refer to the ATSDR Toxicological Profile for Guthion, chapter on "Health Effects" for a more detailed discussion of available information (ATSDR 2008). Generally, ATSDR believes that the most relevant route of human exposure to guthion at waste sites is oral, thus ATSDR scientists believe that the proposed toxicity studies should be conducted via the oral route. Additionally, animal testing should be conducted on the species with metabolism most similar to humans or the most sensitive species.

1. Levels I & II Data Needs

ATSDR determines Minimal Risk Levels (MRLs) which are defined as estimates of daily human exposure to a chemical that are likely to be without appreciable risk of deleterious effects over a specified duration. In order to derive MRLs for acute, intermediate, and chronic exposure durations, ATSDR evaluates the substance-specific database to identify studies of the appropriate route and duration of exposure. Thus, in order to derive acute MRLs, ATSDR evaluates studies of 14 days or less duration that identify the target organs and levels of exposure associated with these effects. Similar studies are identified for intermediate and chronic duration exposures.

Currently, ATSDR is using tools such as physiologically-based pharmacokinetic modeling and pharmacodynamic modeling to extrapolate data across routes or durations of exposure. ATSDR acknowledges that such extrapolations may be done on a substance-by-substance basis after adequate toxicokinetics information has been collected.

As reflected in the Decision Guide, ATSDR assigns priorities to identified data needs for acute/intermediate (Level I) studies by the most relevant route of exposure at Superfund sites. Regarding the need to conduct studies by other routes of exposure, ATSDR usually first requires toxicokinetic studies for the three routes of exposure to determine the need for the additional route-specific information.

Regarding chronic studies, ATSDR acknowledges that appropriately conducted 90-day studies can generally predict the target organs for chronic exposure. However, they might fall short in accurately predicting the levels of exposure associated with these effects. Although ATSDR

acknowledges this fact, it will generally await the results of prechronic and toxicokinetic studies before assigning priority to chronic toxicity studies. Note: Chronic toxicity studies may be separated from cancer bioassays; they require a one-year exposure.

a. Acute-Duration Exposure

Purpose: To determine whether adequate data exist to identify target organs and levels of exposure that present a significant risk to cause acute human health effects.

Finding: A data need to conduct additional studies via inhalation, oral, and dermal exposure has been identified. The available studies indicate that the nervous system is the target organ of toxicity for guthion via the inhalation, oral, and dermal routes. No studies were available of acute-duration inhalation exposures in humans. Two studies have examined the acute inhalation toxicity of guthion in animals (EPA 1978; Kimmerle 1976). Both studies identify inhibition of acetylcholinesterase activity as the most sensitive end point. The 25% reduction in erythrocyte acetylcholinesterase activity, which was not associated with changes in appearance or behavior, was selected as the basis of an acute-duration inhalation MRL. The study by Kimmerle (1976) also identified a no-observed-adverse-effect level (NOAEL) for reductions in acetylcholinesterase activity. Although the selection of the critical effect is well supported by longer-duration inhalation studies and oral exposure studies, the available acute-inhalation studies did not examine a wide variety of possible targets. A data need exists for a comprehensive assessment of the toxicological effects of inhaled guthion in additional animal species.

No studies were available of acute-duration oral exposures in humans. Studies in animals indicate that reductions in brain and erythrocyte cholinesterase activity in rats (Astroff and Young 1998; Pasquet et al. 1976) were the most sensitive end points affected upon acute-duration oral exposure to guthion. Rats administered single (Pasquet et al. 1976) or repeated doses (Astroff and Young 1998) of guthion showed reductions in brain and erythrocyte acetylcholinesterase activity as high as 40 and 75%, respectively. In rats treated with single (EPA 1978) or repeated (Short et al. 1980) oral doses of guthion, clinical signs of neurotoxicity (salivation, lacrimation, defecation, urination, exophthalmus, tremors, and muscle fasciculations) were observed at oral doses that were 4–8 times higher than the lowest doses associated with reductions in brain and erythrocyte cholinesterase activity (Astroff and Young 1998; Pasquet et al. 1976). Tremors, salivation, urination, and lacrimation were also observed in mice administered repeated oral doses

of guthion (Short et al. 1980). In some of the studies with rats and mice, increased mortality was observed in animals that showed the clinical signs described above (Short et al. 1980). Reductions in the incidence of viable litters and fetal body weight were observed in pregnant mice at single oral doses also associated with reductions in maternal weight gain and increased maternal mortality (Kavlock et al. 1985). Reductions in maternal body weight gain were also observed in rats treated orally with guthion during gestation (Short et al. 1980). Increases in the incidence of supernumerary ribs (Kavlock et al. 1985) and malaligned sternebrae (Short et al. 1980) were observed in the offspring of mice administered sublethal doses of guthion orally during gestation. The studies by Astroff and Young (1998) and Pasquet et al. (1976) identified the lowest lowest-observed-adverse-effect levels (LOAELs) for significant reductions in erythrocyte and brain cholinesterase activity in rats; however, only Astroff and Young (1998) identified a NOAEL. Thus, the study by Astroff and Young (1998) was used to derive an acute-duration oral MRL. Although the available data are sufficient to derive an acute-duration oral MRL, there is a data need to conduct a comprehensive assessment of the systemic toxicity of guthion after acute-duration oral exposure in more than one animal species.

Studies in humans indicate that allergic reactions to guthion applied on the skin are exceedingly rare (Sartorelli et al. 1999) or non-existent (Lisi et al. 1987). Comprehensive toxicological assessments in humans after acute-duration dermal exposures to guthion were not available. Agricultural workers exposed to guthion, presumably via dermal exposure, showed reductions in erythrocyte cholinesterase, but did not show clinical signs of guthion intoxication (Franklin et al. 1981; McCurdy et al. 1994; Schneider et al. 1994). Dermal exposure studies in rats (EPA 1978; Gaines 1960; Pasquet et al. 1976) and mice (Skinner and Kilgore 1982) have only evaluated neurological effects and lethality. Reductions in erythrocyte cholinesterase activity were observed in mice (Skinner and Kilgore 1982) and clinical signs of intoxication (salivation, lacrimation, exophthalmus, defecation, urination, and muscle fasciculations) were observed in rats (EPA 1978) after acute-duration dermal exposures to guthion. There is a need to conduct additional studies to establish the toxic effects of dermal exposure to guthion.

Priority Recommendation: The identified data need to conduct additional studies via inhalation exposure is not considered priority because the available data were sufficient to derive an acute-duration MRL and inhalation exposure is not a primary route of exposure at hazardous waste sites. The identified data need to conduct additional studies via oral exposure is not considered priority because the available data were sufficient to derive an acute-duration oral MRL. The

identified data need to conduct additional dermal toxicity studies is not considered priority because dermal exposure to guthion is not a primary exposure route at hazardous waste sites.

b. Intermediate-Duration Exposure

Purpose: To determine whether adequate data exist to identify target organs and levels of exposure that present a significant risk to cause subchronic human health effects.

Finding: A data need to conduct additional studies via inhalation, oral, and dermal exposure has been identified. The available studies indicate that the nervous system is the target organ of toxicity for guthion via the inhalation and oral routes. No studies were available of the effects of intermediate-duration inhalation exposure to guthion in humans. The only available inhalation study in animals noted that there were no changes in the weight or morphology of the lungs, heart, liver, gonads, kidney, thyroid, adrenals, thymus, and spleen in rats (Kimmerle 1976). There also were no significant changes in hematological parameters. Reductions in body weight gain were observed in male rats, but not female rats (Kimmerle 1976). Erythrocyte cholinesterase activity was reduced in male and female rats, but brain cholinesterase activity was not significantly affected and there were no changes in appearance or behavior of the exposed animals (Kimmerle 1976). An intermediate-duration inhalation MRL was derived based on reductions in erythrocyte cholinesterase activity in rats (Kimmerle 1976). Additional intermediate-duration inhalation toxicity studies with more than one animal species are needed in order to establish dose-response relationships.

Data regarding the effects of intermediate-duration oral exposures to guthion in humans are limited to reports available in abstracts. These reports indicate that no significant changes in plasma or erythrocyte cholinesterase activity were observed in a group of five subjects receiving daily oral doses of guthion for 4 weeks (Rider and Puletti 1969; Rider et al. 1970, 1971, 1972). These data are insufficient to establish the dose-response relationship or effects threshold levels of guthion exposure in humans. Studies in animals exposed orally to guthion observed alopecia (Schmidt and Chevalier 1984), reduced body weight gain (Sheets et al. 1997; Vos et al. 1983), unspecified histopathological findings in the pituitary, adrenals, thymus, and testes (Vos et al. 1983), and developmental effects (Holzum 1990; Short et al. 1980) in rats. Gastric effects were reported in dogs exposed orally to guthion but these effects appear to be secondary to guthion neurotoxicity (Allen et al. 1990). Reductions in erythrocyte cholinesterase activity, the most

sensitive end point detected, were observed in rats at 0.55 mg/kg/day (Holzum 1990) and 0.91 mg/kg/day (Sheets et al. 1997) and in dogs at 0.69 mg/kg/day (Allen et al. 1990). Clinical signs of neurotoxicity were also observed in dogs at the lowest adverse effect level, but not in rats exposed to a similar dose; these data suggest that dogs may be more sensitive than rats to the neurotoxic effects of guthion. The dog study (Allen et al. 1990) was used to derive an intermediate-duration oral MRL. A comprehensive assessment of the systemic effects of oral exposure to guthion in more than one species is needed.

No intermediate-duration, dermal exposure studies in humans were available. A dermal exposure study in rabbits reported reductions in body weight gain in females, reductions in red cell count in males, a reduction in erythrocyte cholinesterase activity in both sexes, increased spleen and kidney weight in males, and increased incidence of inflammatory changes in kidneys of males; plasma and brain cholinesterase were unaffected by treatment with guthion (EPA 1999b). Additional studies in more than one animal species are needed in order to conduct a comprehensive assessment of the systemic toxicity of guthion via dermal exposure.

Priority Recommendation: The identified data need to conduct additional studies via inhalation and dermal exposure is not considered priority because inhalation and dermal exposure are not considered to be primary routes of exposure at hazardous waste sites. In addition, the available data were sufficient to derive an intermediate-duration inhalation MRL. The identified data need to conduct additional studies via oral exposure is not considered priority because the available data were sufficient to derive an intermediate-duration oral MRL.

c. Chronic-Duration Exposure

(1) Toxicity Assessment

Purpose: To determine whether adequate data exist to identify target organs and levels of exposure that present a significant risk to cause chronic human health effects.

Finding: A data need to conduct additional studies via inhalation, oral, and dermal exposure has been identified. No inhalation or dermal exposure studies were available in humans or animals and no oral studies in humans were available. The available studies indicate that the nervous system is the target of toxicity for guthion via the oral route. The results of shorter duration

studies suggest that the nervous system might also be the target of toxicity for guthion via the inhalation and dermal exposure routes. The intermediate-duration inhalation MRL (which was based on reductions in erythrocyte cholinesterase activity in rats exposed to inhaled guthion for 12 weeks [Kimmerle 1976]) was adopted for the chronic-duration inhalation MRL. The adoption of the intermediate-duration MRL for the chronic-duration MRL is justifiable given that reductions in erythrocyte cholinesterase activity in rats did not show any biologically significant changes during the 4–12-week observation period (Kimmerle 1976). Moreover, intermediate- and chronic-duration oral exposure studies in rats (Schmidt and Chevalier 1984) and in dogs (Allen et al. 1990) suggest that there are no duration-dependent increases in the severity of the inhibition of erythrocyte cholinesterase activity. Additional chronic-duration inhalation exposure studies in more than one animal species are needed to conduct a comprehensive assessment of the toxicity of guthion and to establish dose-response relationships for chronic-duration inhalation exposures.

Three chronic toxicity studies in animals administered guthion in the diet are available. A toxicity assessment in rats administered guthion in the diet for 2 years revealed reductions in body weight gain, elevated thrombocyte values, and increased incidence of alopecia. Rats did not show increased mortality or evidence of hepatic, renal, or ocular effects (Schmidt and Chevalier 1984). A study in dogs administered guthion in the diet for 1 year did not find any evidence of ocular, hematological, hepatic, or renal effects at a dose that elicited reductions in body weight gain (Allen et al. 1990). An increased incidence of mucoid diarrhea and emesis observed in male and female dogs was attributed to the neurotoxic effect of guthion (Allen et al. 1990). Reductions in erythrocyte cholinesterase activity were observed in rats at 0.75 mg/kg/day (Schmidt and Chevalier 1984) and dogs at 0.69 mg/kg/day (Allen et al. 1990). Non-neoplastic lesions were examined in rats and mice administered guthion in the feed for 80 weeks during a carcinogenicity assay (NCI 1978). A variety of non-neoplastic lesions were observed among control and dosed rats and mice; these lesions were not considered to be related to guthion exposure. There was, however, an increase in the incidence of cystic endometrial hyperplasia in female mice administered 10.8 mg/kg/day (NCI 1978). Thus, although three chronic-duration oral exposure studies were available, there is a data need to evaluate the role of guthion in the increased incidence of cystic endometrial hyperplasia in female mice (NCI 1978). A chronic-duration oral MRL was derived based on the inhibition of erythrocyte cholinesterase activity in male dogs after 52 weeks (Allen et al. 1990). The reductions in erythrocyte cholinesterase activity and the effects associated with it were the most sensitive end points of guthion toxicity (Allen et al. 1990).

There is a data need for chronic-duration dermal exposure studies in more than one animal species in order to conduct a comprehensive toxicity assessment of guthion.

Priority Recommendation: The identified data need to conduct additional studies via inhalation, oral, and dermal exposure is not considered priority. Inhalation and dermal exposures are not considered to be primary exposure routes to guthion at hazardous waste sites and the available data were sufficient to derive chronic-duration inhalation and oral MRLs.

(2) Cancer Assessment

Purpose: To determine whether populations potentially exposed to guthion are at an increased risk for developing cancer for purposes of conducting meaningful follow-up exposure and health studies. Similar to toxicity end point assessment, when bioassays are indicated because of the potential for substantial exposure and the lack of information on carcinogenicity, ATSDR will generally only assign priority to a bioassay conducted via the most relevant route of human exposure at Superfund sites.

Comparative toxicokinetic information across routes as previously discussed will be assigned priority and conducted before assigning priority to any additional routes of exposure. In cases where the assessment of chronic toxicity and carcinogenicity can be combined, they will.

Finding: A data need to conduct additional studies of the carcinogenic potential of guthion via inhalation, oral, and dermal exposure has been identified. No carcinogenicity studies in humans or animals following inhalation or dermal exposure and no studies in humans exposed orally were identified. Thus, there is a need to conduct carcinogenicity studies using two animal species to adequately assess the potential carcinogenicity of guthion via inhalation and dermal routes of exposure. The carcinogenicity of guthion was evaluated in a study in rats and mice administered guthion in the diet for 80 weeks followed by several weeks of observation (NCI 1978). Under the conditions of the bioassay, NCI (1978) concluded that guthion was not carcinogenic in male or female mice or female rats. The incidences of neoplasms of the pancreatic islets and of the follicular cells of the thyroid in male rats provide equivocal evidence of the carcinogenic potential of guthion in male rats. Significant increases, relative to pooled controls, were observed in the combined incidence of islet cell adenoma or carcinomas of the pancreas in male rats administered

10.9 mg/kg/day guthion in the diet; however, these carcinomas cannot be clearly implicated to a chemically induced effect because the observed incidence in male rats in this study (13%) falls within the range of the spontaneous incidence of these lesions (incidence of islet cell carcinoma or carcinomas of the pancreas: 0–22% with a mean of 2%) observed in male rats in the conducting laboratory (NCI 1978). Significant increases, relative to pooled controls, were also observed in the incidence of benign thyroid tumors, malignant thyroid tumors, or combined follicular cell tumors in male rats exposed to 5.5 or 10.9 mg/kg/day (NCI 1978); however, the increased incidence of these tumors cannot be clearly implicated to a chemically induced effect because the observed incidence in male rats in this study (32 and 31% in the low and high dose groups, respectively) falls within the range of the spontaneous incidence of these lesions (follicular-cell tumors of the thyroid: 0–43% with a mean of 7%) observed in male rats in the conducting laboratory (NCI 1978). The maximally tolerated dose appears to have been reached in this study as indicated by lower mean body weight (relative to controls) in high dose male and female rats, low dose male rats, and high-dose female mice (NCI 1978). Clinical signs of toxicity observed among treated animals included alopecia, tremors, hyperactivity, hypoactivity, and convulsions. Mortality was not significantly increased in treated rats or mice (NCI 1978). Under current NTP guidelines, the exposure duration for rodent carcinogenicity studies is 2 years, so the NCI (1978) study is limited by a shorter than optimal exposure duration. There was no evidence of the occurrence of treatment-related tumors in male and female rats exposed to 0.25–3.11 mg/kg/day in the diet for 2 years (Schmidt and Chevalier 1984). In 1993, EPA concluded that there was a lack of evidence of carcinogenicity of guthion in male and female mice and rats (EPA 1999). Currently, however, the EPA has no carcinogenicity classification for guthion (IRIS 2006). IARC (IARC 2006) and DHHS (NTP 2005) have not classified guthion as to its carcinogenicity. An additional oral exposure study is needed to clarify the carcinogenic potential of guthion.

Priority Recommendation: The identified data need to conduct additional studies via inhalation and dermal exposure is not considered priority because inhalation and dermal exposure are not considered to be primary routes of exposure at hazardous waste sites. The identified data need to conduct an additional carcinogenicity assay via the oral route is not considered priority because the available database on the carcinogenicity of other organophosphate insecticides does not indicate that these chemicals are carcinogenic.

d. Genotoxicity

Purpose: To evaluate the mechanism of guthion-induced toxicity for purposes of future mitigation activities. Generally, priority is assigned to genotoxicity studies if information is lacking to assess the genotoxic potential of this substance both *in vivo* (mouse micronucleus) and *in vitro* (Ames *Salmonella*). This is particularly true if there are human data to suggest that the substance may act by a genotoxic mechanism to cause cancer, reproductive toxicity, etc., or there exists "structural alerts" that suggest that the substance may be genotoxic. Additional studies will not be assigned priority simply to confirm or refute an equivocal database without justification.

Finding: A data need to conduct additional genotoxicity studies has been identified. No *in vivo* studies of genotoxic effects in humans were located. In the only *in vivo* studies that were located, negative results were reported in a study of recessive lethality in *Drosophila* and two studies of micronuclei formation and dominant lethality in mice (Waters et al. 1982). *In vitro* assays in procaryotic organisms showed negative results in four of five reverse mutation assays (Carere et al. 1978; Hrelia et al. 1990; Waters et al. 1982; Zeiger et al. 1987) and a negative result in one forward mutation assay (Carere et al. 1978). *In vitro* studies with eucaryotic organisms showed positive results for chromosome breaks in two human cell lines (Alam and Kasatiya 1976) and Chinese hamster ovary (CHO) cells (Alam et al. 1974), micronucleus formation in human lymphocytes (Bianchi-Santamaria et al. 1997), forward mutation in mouse lymphoma cells (Waters et al. 1982), and enhanced mitotic recombination (Waters et al. 1982) and crossing over (Hrelia et al. 1990) in *Saccharomyces cerevisiae*. Negative results were observed in assays of gene conversion in *S. cerevisiae* (Waters et al. 1982) and sister chromatid exchange in CHO cells (Chen et al. 1982a, 1982b; Waters et al. 1982), and unscheduled DNA synthesis in human fetal lung fibroblasts (Waters et al. 1982). Additional studies, particularly *in vivo* assays using more than one animal species are needed in order to evaluate the genotoxic risk of guthion in humans.

Priority Recommendation: The identified data need to conduct additional genotoxicity studies is not considered priority because guthion has not been shown to be carcinogenic in studies via oral exposure, the most relevant route of human exposure at waste sites. Genotoxicity assays of a number of organophosphate pesticides have shown mixed results; however, the results of carcinogenicity assays with organophosphate pesticides have been overwhelmingly negative with only a few equivocal results (Storm 2001).

e. Endocrine Disruption

Purpose: To determine whether populations potentially exposed to guthion are at an increased risk to develop toxicity of the endocrine system for purposes of conducting meaningful follow-up exposure and health studies. Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones, or otherwise interfere with the normal function of the endocrine system. Chemicals with this type of activity are most commonly referred to as endocrine disruptors. While there is some controversy over the public health significance of endocrine disrupting chemicals, it is agreed that the potential exists for these compounds to affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior.

Generally, when considering the need to assign priority, in the absence of all information on this end point, ATSDR will assign priority to screening studies that examine effects on a) male and female reproductive organs, and b) other endocrine organs including hypothalamus, pituitary, thyroid, parathyroid, adrenal, pancreas, paraganglia, and pineal body. Such screening level studies include, but are not limited to, *in vitro* studies [e.g., 1) Estrogen Receptor Binding/Transcriptional Activation Assay, 2) Androgen Receptor Binding/Transcriptional Activation Assay, and 3) Steroidogenesis Assay with Minced Testis], and *in vivo* studies [e.g., 1) Rodent 3-day Uterotropic Assay, 2) Rodent 20-day Pubertal Female Assay with Thyroid, 3) Rodent 5–7-day Herschberger Assay].

If any of the following is true, then ATSDR will consider assigning Level II priority to 2-generation reproductive studies: if (1) there are suggestions that guthion may have endocrine disrupting potential from Level I studies; or (2) if there have been human anecdotal reports of endocrine disrupting effects following guthion exposure; or (3) if there are structurally similar compounds that affect the endocrine system.

As before, priority will be assigned to studies conducted by the most relevant route of human exposure at Superfund sites; comparative toxicokinetic studies will be performed and evaluated before assigning priority to studies conducted via additional routes of exposure.

Finding: A data need to conduct studies of the effect of guthion on the endocrine system via inhalation, oral, and dermal route of exposure has been identified. No human or animal data on the potential of guthion to induce effects indicative of endocrine disruption were identified. A chronic-duration study (NCI 1978) found an increased incidence of cystic endometrial hyperplasia in female mice administered guthion in the diet. An intermediate-duration oral study reported unspecified histopathological alterations in the pituitary, adrenals, and testes (Vos et al. 1983). However, it is not known if these effects are due to direct damage to the endocrine tissue or whether the effects are mediated through the neuroendocrine axis. The available animal developmental and reproductive toxicity studies (Holzum 1990) did not find effects suggestive of endocrine disruption. *In vivo* and *in vitro* screening level endocrine disruption studies are needed in order to assess the endocrine disruption potential of guthion.

Priority Recommendation: The identified data need to conduct additional studies on the endocrine system is not considered priority. The available data provide marginal evidence that the endocrine system may be a target of toxicity; however, the mechanisms of the effects are not known. The recommended *in vitro* and *in vivo* endocrine disruption screening studies, as well as the recommended chronic toxicity, developmental toxicity, and reproductive toxicity studies conducted via oral exposure should provide sufficient information to evaluate the sensitivity of this end point.

f. Reproductive Toxicity

Purpose: To determine whether populations potentially exposed to guthion are at an increased risk to develop reproductive effects for purposes of conducting meaningful follow-up exposure and health studies. ATSDR scientists believe it is important to acquire reproductive toxicity data in order to consider the needs of susceptible populations. It is desirable to have information on reproductive toxicity before developing MRLs to ensure that target organs have been adequately evaluated.

Generally, when considering the need to assign priority, in the absence of all information on this end point, ATSDR will assign priority to the conduct of 90-day studies with special emphasis on reproductive organ pathology. If any of the following is true, then ATSDR will consider assigning priority to multigeneration animal studies: (1) If any indication is found in these studies that the reproductive system of either male or female animals is a target organ of

substance exposure; or (2) if there have been human anecdotal reports of reproductive effects following substance exposure; or (3) if there are structurally similar compounds that affect reproduction.

As before, priority will be assigned to studies conducted by the most relevant route of human exposure at Superfund sites; comparative toxicokinetic studies will be performed and evaluated before assigning priority to studies conducted via additional routes of exposure.

Finding: A data need to conduct additional reproductive studies via inhalation, oral, and dermal exposure has been identified. No studies are available on the reproductive toxicity of guthion in humans through any route of exposure or in animals exposed by inhalation or dermal contact. Insemination, fertility, or gestation indices or duration of gestation were not affected in male or female rats administered guthion in the diet before mating and continuously through gestation (Holzum 1990). Unspecified histopathologic findings were observed in the testes of rats administered guthion in the diet for 3 weeks; however, increased mortality was also observed at that dose (Vos et al. 1983). A histopathologic examination of the reproductive organs of rats and mice conducted at termination of a carcinogenicity study reported non-neoplastic lesions that were similar to those observed in aging rats and mice; however, an increase in the incidence of cystic endometrial hyperplasia was observed in female mice (NCI 1978). There is a data need to conduct additional reproductive toxicity studies in more than one animal species via inhalation, oral, and dermal exposure in order to determine if guthion can adversely alter reproductive end points.

Priority Recommendation: The identified data need to conduct additional reproductive toxicity studies via the inhalation, oral, and dermal exposure route is not considered priority because the available data from oral studies in animals do not suggest that guthion directly elicits adverse reproductive effects. In addition, inhalation and dermal exposure are not considered primary routes of exposure at hazardous waste sites.

g. Developmental Toxicity

Purpose: To determine whether populations potentially exposed to guthion are at an increased risk for developmental effects for purposes of conducting meaningful follow-up exposure and

health studies. Similar to reproductive toxicity assessment, Agency scientists believe it is important to assess the developmental toxicity data.

In the absence of any reproductive or teratologic information, ATSDR will consider proposals to simultaneously acquire reproductive and teratological information. ATSDR acknowledges that, in some circumstances, developmental studies may be assigned priority if the following statements are true: (1) if a two-generation reproductive study provides preliminary information on possible developmental toxicity of guthion, (2) if there are human anecdotal reports of developmental effects following guthion exposure, *or* (3) if structurally similar compounds have caused developmental effects.

As for reproductive toxicity, priority will be assigned to studies conducted by the most relevant route of human exposure at Superfund sites; comparative toxicokinetic studies will be performed and evaluated before assigning priority to the conduct of studies via additional routes of exposure.

Finding: A data need to conduct additional developmental studies via inhalation, oral, and dermal exposure has been identified. A study of male agricultural workers found no association between occupational exposure to guthion and the occurrence of congenital malformations in their children (García et al. 1998). Additional studies on the developmental toxicity of guthion in humans by any route of exposure are not available. Significant reductions in brain weight and brain acetylcholinesterase activity were observed in 5-day-old pups from female rats administered guthion in the feed at 4.9 mg/kg/day, but not in rats fed 1.5 mg/kg/day from mating through lactation (Holzum 1990). Brain acetylcholinesterase activity was further reduced in 28-day-old pups, but brain weight was not different from that in control animals. No treatment-related malformations were observed in these animals; additional developmental end points were not assessed (Holzum 1990). Clinical signs, such as stiff rear legs, lack of neuromuscular coordination, and tremors (Short et al. 1980) were observed in pups of pregnant rats administered guthion orally. Acetylcholine, acetylcholinesterase, and butyrylcholinesterase are involved in the development of the nervous system (Brimijoin and Koeningsberger 1999; Layer 1990; Layer and Willbold 1994) and some of this development is not completed until adulthood. It is plausible then that by interfering with the normal function and levels of these neurotransmitters and enzymes during development, guthion might elicit adverse developmental effects in the nervous system. An increased incidence of supernumerary ribs and reduced fetal body weight gain were observed in the offspring of pregnant mice administered an oral dose of guthion (Kavlock et al.

1985). An increased incidence of malaligned sternbrae was observed in fetuses of pregnant mice administered doses that were also maternally neurotoxic (Short et al. 1980). Although some of the developmental effects were observed at doses that were maternally toxic, it cannot be established whether or not the developmental effects were an indirect result of maternal toxicity. There is a need to conduct additional inhalation, oral, and dermal studies in two animal species in order to comprehensively evaluate the developmental toxicity of guthion.

Priority Recommendation: The identified data need to conduct additional developmental toxicity studies via oral exposure is considered priority. It has been shown that acetylcholine, acetylcholinesterase, and butyrylcholinesterase are involved in the development of the nervous system (Brimijoin and Koeningsberger 1999; Layer 1990; Layer and Willbold 1994) and some of this development is not completed until adulthood. As has been demonstrated, acetylcholinesterase activity is highly sensitive to guthion. It is plausible then that by interfering with the normal function and levels of these neurotransmitter and enzymes at critical periods during development, guthion might elicit adverse developmental effects in the nervous system. It would be useful for future developmental studies to include a pharmacokinetic evaluation to determine the distribution of guthion in the pregnant animal and exposure levels to the fetus. Developmental effects have been observed in animals exposed to other organophosphate pesticides. For instance, pups of pregnant mice administered diazinon (0.18 or 9 mg/kg/day) orally throughout gestation showed endurance and coordination deficits in neuromuscular function tests (Spyker and Avery 1977). Morphological abnormalities were observed in the forebrain area of pups from dams administered 9 mg/kg/day but not pups from dams in the 0.18 mg/kg/day group. Dams from either dose group showed reduced weight gain (Spyker and Avery 1977). Significant treatment-related reductions in the measurement of the parietal cortex and possible alterations in the hippocampal gyrus were observed on postnatal day 66 in the brain of female rats from dams administered chlorpyrifos at 1 mg/kg/day from gestation day 6 through lactation day 11 (EPA 2000b). Reductions in maternal plasma and erythrocyte ChE were the only effects observed at this dose. Thus, additional studies are needed to conduct a comprehensive evaluation of the neurodevelopmental effects of guthion. The identified data need to conduct additional developmental toxicity studies via the inhalation and dermal exposure routes is not considered priority because inhalation and dermal exposure are not considered primary routes of exposure at hazardous waste sites.

h. Immunotoxicity

Purpose: To evaluate the mechanism of guthion-induced toxicity for purposes of defining target organs and future mitigation activities. There is evidence to suggest that the immune system might be a susceptible target organ for many environmental contaminants. In the absence of any information on the immune system as a target organ, priority will be assigned to the evaluation of the immune system (lymphoid tissue, blood components) as an end point in 90-day studies (Level I) before assigning priority to an immunotoxicology battery as recently defined by the NTP.

For those substances that either (1) show evidence of immune system effects in 90-day studies, (2) have human anecdotal data to suggest that the immune system may be affected, or (3) are structurally similar to known immunotoxicants, an immunotoxicology battery of tests will be assigned priority.

Finding: A data need to conduct additional immunotoxicity studies via inhalation, oral, and dermal exposure has been identified. No studies were located of the immune toxicity of guthion in humans exposed by inhalation or oral routes. Two studies examined the incidence of allergic responses in volunteers who were applied patches containing guthion on the skin. These studies show that immune responses to dermal applications of guthion are exceedingly rare (Lisi et al. 1987; Sartorelli et al. 1999). Thymus and spleen morphology were not affected in rats exposed to nonlethal concentrations of guthion by inhalation for up to 12 weeks (Kimmerle 1976). Decreased relative weight of the spleen and mesenteric lymph nodes and unspecified histopathologic findings in the thymus were observed in male rats administered guthion in the diet for 3 weeks at doses that also elicited increased mortality (Vos et al. 1983). Histopathologic assessments conducted at termination of a carcinogenicity study in rats and mice did not show treatment-related effects on the spleen or lymph nodes (NCI 1978). There is a data need to conduct studies of the immunotoxicity of guthion in more than one animal species. There are no available studies that assessed immune function in animals exposed to guthion via inhalation, oral, or dermal routes.

Priority Recommendation: The identified data need to conduct additional immunotoxicity studies via the oral route is not considered priority because currently there are no data to indicate that guthion elicits immunotoxic effects. The identified data need to conduct additional immunotoxicity studies via inhalation and dermal routes of exposure is not considered priority

because inhalation and dermal exposures are not the primary routes of exposure to guthion at hazardous waste sites.

i. Neurotoxicity

Purpose: To evaluate the mechanism of guthion-induced toxicity to define target organs and future mitigation activities. Similar to immunotoxicity, there is a growing body of data to suggest that the nervous system is a very sensitive target organ for many environmental chemicals. In the absence of any information on the nervous system as a target organ, priority will be assigned evaluation of the nervous system as an end point in 90-day studies (Level I) before assigning priority to a neurotoxicology battery.

It may be possible to assign priority to evaluation of demeanor in 90-day studies along with neuropathology. For those substances that either (1) show evidence of nervous system effects in 90-day studies, (2) have human anecdotal data to suggest that the nervous system may be affected, or (3) are structurally similar to known neurotoxicants, a neurotoxicology battery of tests will be assigned priority.

Finding: A data need to conduct additional neurotoxicity studies via inhalation, oral, and dermal exposure has been identified. The available studies indicate that the nervous system is the target organ of toxicity of guthion regardless of the route of exposure. No significant changes in plasma or erythrocyte cholinesterase activity were observed in a small group of subjects who took guthion orally for 4 weeks (Rider and Puletti 1969; Rider et al. 1970, 1971, 1972); however, studies of agricultural workers have observed reductions in erythrocyte or whole blood cholinesterase activity after applying guthion (Franklin et al. 1981) or after entering fields treated with guthion (Kraus et al. 1977; McCurdy et al. 1994; Schneider et al. 1994). Despite these reductions in cholinesterase activity, workers did not exhibit clinical signs of neurotoxicity. Reductions in erythrocyte, brain, plasma, or whole blood cholinesterase activity or clinical signs of neurotoxicity have been observed in rats exposed to guthion by inhalation (EPA 1978; Kimmerle 1976), in rats (Astroff and Young 1998; EPA 1978; Holzum 1990; Pasquet et al. 1976; Schmidt and Chevalier 1984; Sheets et al. 1997; Short et al. 1980; Su et al. 1971), mice (Short et al. 1980), and dogs (Allen et al. 1990) exposed orally, and in rats (EPA 1978) and mice (Skinner and Kilgore 1982) exposed dermally. No data are currently available to assess the potential long-term neurological effects of intermittent exposures to guthion. Such information might be

obtained by administering tests designed to detect subtle neurological effects among workers exposed to guthion or in animal studies.

Priority Recommendation: The identified data need to conduct additional studies via the oral route of exposure is not considered priority because there is no evidence to indicate that chronic exposure to guthion may result in neurological effects other than those that have been identified in the available acute-, intermediate-, and chronic-duration studies. The identified data need to conduct additional studies via inhalation and dermal routes are not considered priority because these are not primary routes of exposure to guthion at hazardous waste sites.

j. Toxicokinetics

Purpose: To evaluate the disposition of guthion across species and routes of exposure to elucidate target organs and mechanisms of toxicity, and to assess the need to conduct studies by routes other than the primary route of exposure.

Finding: A data need to assess the toxicokinetics of guthion following inhalation, oral, and dermal exposure has been identified. No studies are available of the absorption, distribution, metabolism, or excretion of guthion in humans or animals following inhalation exposure. The observed reductions in erythrocyte (Kimmerle 1976) and whole blood (EPA 1978) cholinesterase activity in rats exposed to guthion aerosols indicate that guthion is absorbed via the inhalation pathway. There are no available human data to estimate the absorption of guthion in humans after oral exposure. In rats administered guthion orally, the radiolabeled guthion residues were eliminated in exhaled air, feces, and urine, but guthion and its oxon metabolite, gutoxon, were not detected in urine (Fakhr et al. 1996). Guthion metabolites have been detected in the urine after dermal application of guthion to humans (Feldmann and Maibach 1974; Franklin et al. 1986) and rats (Franklin et al. 1983). Approximately 16% of a dermal dose of radiolabeled guthion applied to volunteers was eliminated in urine within 120 hours (Feldmann and Maibach 1974), but approximately 60% of a dermal dose applied to rats was recovered in urine as a guthion metabolite (Franklin et al. 1983). The dermal absorption of guthion in rats 168 hours after application ranged from 18.3 to 41.7% and was inversely related to the applied dose (Zendzian 2003). No studies were located that directly evaluate the comparative toxicokinetics of guthion in animals and humans. Nevertheless, available studies indicate that neural acetylcholinesterase is the target organ of toxicity for guthion in animals and humans (Buratti et al. 2003; Hitchcock and

Murphy 1971). The bioactivation and detoxication of guthion has been described (Dahm et al. 1962; Hitchcock and Murphy 1971; Levine and Murphy 1977; Motoyama and Dauterman 1972; Sultatos and Woods 1988). Details regarding the identity and affinities of three cytochromes involved in the bioactivation of guthion in the human liver have been published (Buratti et al. 2003). No studies are available to determine if the activities of these cytochromes in humans differ from that in animals. Although conducting additional toxicokinetic studies in animals exposed to guthion via the inhalation, oral, and dermal routes might be useful, the available oral study in rats is sufficient to indicate that guthion is absorbed and distributed to internal organs and eliminated in urine, feces, and exhaled air (Fakhr et al. 1996).

Priority Recommendation: The identified data need to assess the toxicokinetics of guthion following inhalation, oral, and dermal exposure is not considered priority. Additional studies would be useful to establish whether there are differences in the toxicokinetics of guthion across species; however, the available oral study in rats is sufficient to indicate that guthion is absorbed and distributed to internal organs and eliminated in urine, feces, and exhaled air (Fakhr et al. 1996). Inhalation and dermal studies are not considered priority because these routes of exposure are not considered the primary exposure routes for individuals living at hazardous waste sites.

2. Level III Data Needs

a. Epidemiologic Studies

Purpose: To evaluate the extant epidemiologic database and to propose the conduct of additional studies that may lead to cause- and effect- findings. The ATSDR Division of Health Studies will be informed of all candidate substances.

Finding: A data need has been identified. Data regarding the effects of oral exposures to guthion in humans are limited to reports available in abstracts. No significant changes in plasma or erythrocyte cholinesterase activity were observed in a group of five subjects receiving daily oral doses of guthion for 4 weeks (Rider and Puletti 1969; Rider et al. 1970, 1971, 1972). These data are insufficient to establish the dose-response relationship or effects threshold levels of guthion exposure in humans. One available study in workers reported an association between the occurrence of systemic illnesses (defined as an acute illness following pesticide exposure, with symptoms and signs not restricted to the eyes or skin) in workers and agricultural use of guthion

(Weinbaum et al. 1997). No association was observed between the occurrence of birth defects (nervous system defects, cardiovascular defects, oral clefts, epispadia or hypospasia, and musculoskeletal defects) and occupational exposure of fathers to guthion (García et al. 1998). Assessments have been conducted of agricultural workers who applied guthion (Franklin et al. 1981) or entered fields treated with guthion (Kraus et al. 1977; McCurdy et al. 1994; Schneider et al. 1994); however, these studies have been limited to the examination of changes in erythrocyte cholinesterase activity over brief exposure durations and have generally not addressed systemic effects. Studies in humans indicate that allergic reactions to guthion applied on the skin are exceedingly rare (Sartorelli et al. 1999) or non-existent (Lisi et al. 1987). There are no studies that examine the existence of subtle, long-term effects of guthion exposure in humans. An epidemiologic study in workers exposed chronically to guthion could be useful in that regard; however, an accurate quantification of exposure to guthion would be necessary to derive useful data from such a study, particularly given that exposure to multiple chemicals is likely.

Priority Recommendation: The identified data need to conduct epidemiologic studies in persons exposed chronically to guthion is not considered priority. The results of epidemiologic studies of exposed populations such as agricultural workers or populations living near hazardous waste sites would be confounded by the concurrent or serial exposure to multiple chemicals; however, an epidemiologic study might be considered if a population with well-documented exposures to guthion alone is identified.

b. Mechanism of Toxic Action

Purpose: To evaluate the mechanism of guthion-induced toxicity to define target organs and future mitigation activities.

Finding: A data need has not been identified. Guthion is an organophosphate insecticide and the mechanism of toxicity of organophosphate insecticides has been extensively studied and described. Studies in humans (Franklin et al. 1981; Kraus et al. 1977; McCurdy et al. 1994; Schneider et al. 1994) and in animals (Allen et al. 1990; Astroff and Young 1998; EPA 1978; Holzum 1990; Kimmerle 1976; Pasquet et al. 1976; Sheets et al. 1997; Short et al. 1980) indicate that the most consistent and sensitive systemic effects of exposure to guthion are related to its direct effect on the nervous system and the secondary effects that result from it. Guthion exerts its systemic effects through inhibition of cholinesterases, specifically acetylcholinesterase in the

central and peripheral nervous system. Acetylcholinesterase is also present in erythrocytes and its activity is commonly used as a surrogate indicator of the effect on neural acetylcholinesterase activity. Guthion is bioactivated *in vivo* and *in vitro* to its oxygen analog form, variably referred to as gutoxon or azinphos-methyl oxon (Buratti et al. 2003; Hitchcock and Murphy 1971; Sultatos and Woods 1988). Gutoxon reacts with a serine hydroxyl group at the active site of acetylcholinesterase, rendering it largely inhibited and unreactive. Under normal circumstances, acetylcholinesterase rapidly and efficiently degrades the neurotransmitter acetylcholine following its release at the nerve synapse or at a neuromuscular junction; however, the inhibited acetylcholinesterase enzyme cannot degrade acetylcholine and the neurotransmitter accumulates at the ending of cholinergic nerves with the ensuing continual stimulation of electrical activity (Carrier and Brunet 1999). Cholinergic nerves play an important role in the normal function of the neuromuscular, central nervous, endocrine, immunological, and respiratory systems (Carrier and Brunet 1999). Thus, the inhibition of the enzyme acetylcholinesterase by gutoxon may have profound and wide-ranging systemic effects. Acetylcholine can be found in the autonomic nervous system, the somatic motor nervous system, and the central nervous system. In the autonomic nervous system, accumulation of acetylcholine would lead to the overstimulation of the muscarinic receptors of the parasympathetic nervous system, which would lead to effects on the exocrine glands (increased salivation, perspiration, lacrimation), eyes (miosis, blurred vision), gastrointestinal tract (nausea, vomiting, diarrhea), respiratory system (excessive bronchial secretions, wheezing, and tightness of chest), and cardiovascular system (bradycardia, decrease in blood pressure) (Ecobichon 1995). Stimulation of the nicotinic receptors in the parasympathetic or sympathetic nervous system of the autonomic nervous system would also lead to effects on the cardiovascular system such as tachycardia, pallor, and increased blood pressure. In the somatic nervous system, nerve fibers innervate the skeletal muscles motor end-plates. Accumulation of acetylcholine in the somatic nervous system would affect skeletal muscle and would manifest itself as muscle fasciculations, cramps, paralysis, and flaccid or rigid tone, among other signs and symptoms. Overstimulation of the nerves in the central nervous system, specifically the acetylcholine receptors of the brain, by the accumulation of acetylcholine may result in lethargy, drowsiness, and mental confusion among other effects. More severe effects on the central nervous system include a state of coma without reflexes, depression of the respiratory centers, and cyanosis (Ecobichon 1995). It has been recognized that, after repeated exposures to organophosphate insecticides, humans and other animal species may develop tolerance to the appearance of cholinergic signs (Costa et al. 1982). It has been proposed that this tolerance to the effect of excess acetylcholine develops by the down-regulation of postsynaptic cholinergic

receptors. This reduces the apparent cholinergic symptoms even in the presence of marked reductions in erythrocyte acetylcholinesterase activity (Sultatos 1994). Other esterases, such as carboxylesterase, may be involved in the toxicity of organophosphate insecticides. For instance, malaoxon, the oxon form of malathion, is hydrolyzed by a carboxylesterase. When the carboxylesterase is inhibited, the acute toxicity of malaoxon increases (ATSDR 2003); however, no data were located that indicate what role carboxylesterases may play in the toxicity of guthion.

Priority Recommendation: A data need has not been identified. The mechanism of toxic action of guthion has been extensively described.

c. Biomarkers

Purpose: To evaluate the need to develop additional biomarkers of exposure and effect for purposes of future medical surveillance that can lead to early detection and treatment.

Finding: A data need has been identified. It has been shown that dimethyl dithiophosphate (DMDTP), dimethyl thiophosphate (DMTP), and dimethyl phosphate (DMP) are metabolic products of the *in vivo* metabolism of guthion (Carrier and Brunet 1999). DMTP was detected in the urine of volunteers 72 hours after they received an application of guthion on the forehead (Franklin et al. 1986). Urinary excretion of the metabolites DMDTP, DMTP, and DMP was detected in a group of individuals not known to be exposed occupationally to guthion (Aprea et al. 1994). Although detection of DMDTP, DMTP, and DMP may be suggestive of exposure to guthion, these metabolites can also be detected after exposure to other organophosphate insecticides. Thus, monitoring for DMP, DMTP, and DMDTP provides information regarding the potential exposure to organophosphate pesticides in general. Neither guthion nor gutoxon were detected in urine in rats administered an oral dose of guthion (Fakhr et al. 1996). No studies were located that detected guthion or gutoxon in blood of exposed animals or humans. Currently, there are no biomarkers for the quantification of exposure to guthion specifically. Monitoring erythrocyte or plasma cholinesterase activity may assist in confirming a diagnosis and perhaps preventing the signs and symptoms of organophosphate poisoning; however, reductions in plasma or erythrocyte cholinesterase activity can be affected not only by all organophosphate insecticides, but also by carbamate ester insecticides. Thus, reductions in cholinesterase activity are not specific to exposure to guthion. In addition, the large degree of variability in cholinesterase activity in human populations (Maroni et al. 2000) indicates that caution should be

exercised when comparing cholinesterase activities from exposed populations, such as agricultural workers, and reference populations. Development of a biomarker of effect specific to guthion would be useful in conducting exposure assessments and epidemiological studies.

Priority Recommendation: Although development of a biomarker specific for guthion would be useful, it is not considered a priority because available monitoring for guthion metabolites and cholinesterase activity are useful indicators of exposure to guthion.

d. Clinical Methods for Mitigating Toxicity

Purpose: To determine whether any efforts are currently under way to mitigate the effects of exposure to guthion.

Finding: A data need has not been identified. Information specific to guthion regarding methods to reduce absorption, body burden, or interfering with the mechanisms of toxic effects was not located. However, information on how to reduce absorption, and body burden, or interfere with the mechanisms of toxic effects of organophosphate pesticides in general is available.

No information was located regarding methods to reduce the absorption of inhaled organophosphate insecticides. Absorption of ingested organophosphate insecticides, and thus, guthion may be reduced by administering activated charcoal (Carlton et al. 1998) or by carefully conducting gastric lavage. Ipecac should not be used for organophosphate poisoning (Osmundsen 1998). A study in rats suggests that the body burden of guthion is expected to be rapidly reduced upon cessation of oral exposure (Fakhr et al. 1996). In cases of dermal exposure, the contaminated area should be promptly washed with copious amounts of soap and water; however, a study in rats indicates that dermal absorption of guthion may continue even after washing the exposed area (Zendzian 2003). Poisoning with organophosphate insecticides is commonly treated by administration of atropine and pralidoxime (2-PAM). Atropine is a competitive antagonist at muscarinic receptor sites and is helpful in drying excessive secretions, especially from the tracheobronchial tree. Glycopyrrolate, a quaternary ammonium compound, has also been used instead of atropine (Bardin and Van Eeden 1990). Nicotinic effects such as muscle weakness and respiratory depression from organophosphate poisoning are commonly treated by administration of 2-PAM, a quaternary amine oxime that can restore enzymatic activity by reversing the phosphorylation of acetylcholinesterase; 2-PAM also has anticholinergic effects

(Carlton et al. 1998). 2-PAM is considered a safe drug with minimal side effects at the recommended antidotal doses (Taylor 2001).

Priority Recommendation: A data need has not been identified given that the currently available methods for mitigating the toxicity of organophosphate insecticides appear to be applicable and adequate for guthion.

e. Children's Susceptibility

Purpose: To determine whether adequate data exist to identify potential health effects from exposures to guthion during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation.

Finding: A data need to conduct additional studies relevant to children's susceptibility via inhalation, oral, and dermal exposure has been identified. There are no human data to determine whether children differ from adults in their susceptibility to the adverse effects of guthion and there are no studies to determine whether there are differences in the pharmacokinetic behavior or metabolism of guthion in children and adults. A study of male agricultural workers found no association between occupational exposure to guthion and the occurrence of congenital malformations (García et al. 1998). Additional studies on the developmental toxicity of guthion in humans by any route of exposure are not available. Adverse developmental effects have been observed in the offspring of rats (Holzum 1990; Short et al. 1980) and mice (Kavlock et al. 1985; Short et al. 1980) administered guthion during gestation. In these studies, developmental effects were observed at doses that were at least as high as those that elicited maternal effects. Guthion shares its mechanism of toxic action with other organophosphate insecticides. Thus, the results of studies conducted with other organophosphate insecticides might shed light on the potential effects that might be observed in children exposed to guthion. Many of the signs of organophosphate poisoning (reductions in plasma and erythrocyte cholinesterase activity, alterations in the function of nervous, cardiac, pulmonary, and gastrointestinal systems, and death) have been observed after acute dermal, inhalation, and oral exposures of children (Dean et al. 1984) and adults (Fazekas 1971; Fazekas and Rengei 1964) to the organophosphate insecticide methyl parathion. These findings suggest that adults and children share similar targets of toxicity

from exposure to organophosphate insecticides. These findings might apply to guthion given the similarities in the mechanism of action between the two pesticides. No reports of poisonings of children exposed to guthion were located. The neurotoxicity of guthion is dependent on its bioactivation to the oxon form via cytochrome P450 (Buratti et al. 2003). Recent work suggests that the bioactivation of guthion in human liver microsomes proceeds via two steps involving more than one cytochrome characterized by different affinities (Buratti et al. 2003). It has been observed that some P450 isozymes are regulated differently during development than during adulthood (Leeder and Kearns 1997). Thus, although sufficient information specific to guthion is not available, it is reasonable to conclude that developmental differences in the regulation of P450 isozymes in children and adults could lead to differences in the bioactivation and resulting toxicity of guthion. Acetylcholine, acetylcholinesterase, and butyrylcholinesterase are involved in the development of the nervous system (Brimijoin and Koenigsberger 1999; Layer 1990; Layer and Willbold 1994) and some of this development is not completed until adulthood. It is plausible then that by interfering with the normal function and levels of these neurotransmitter and enzymes during development, guthion might elicit adverse developmental effects in the nervous system. Erythrocyte acetylcholinesterase activity increases with age, starting at birth until >60 years of age (Garcia-Lopez and Monteoliva 1988). It is not known whether these changes in activity might elicit different responses to guthion among children and adults. Currently, there are no validated biomarkers of exposure or effect to be evaluated in children or in adults who were exposed to guthion during childhood. Additional studies are needed to evaluate potential age-specific differences in toxicity and toxicokinetics of guthion and the long-term effects of *in utero* exposure to guthion.

Priority Recommendation: The identified data need to conduct additional studies on children's susceptibility via inhalation, oral, and dermal route is not considered priority. There is no preliminary evidence to suggest that children may handle guthion differently from adults and there are no reports of toxic effects in children following exposure to guthion.

IV. Summary: Prioritization of Data Needs for guthion

A. Exposure

Application of the hierarchy of research priorities presented in the Decision Guide begins with the evaluation of available analytical methods for guthion and proceeds through assessing the need

for epidemiologic studies. As stated previously, much information is available on guthion, though some of the studies are very old. This does not mean that data derived from older studies are not adequate. ATSDR agrees with the National Research Council in that it is not appropriate to judge the quality of past and future studies solely by the standards of today.

Building a sound basic data foundation for higher level environmental research via the Decision Guide requires the determination of human exposure levels and media-specific data on guthion. Although a lot of information is available, a need to evaluate existing data on concentrations of guthion in contaminated environmental media at hazardous waste sites has been identified.

Furthermore, a need to collect data on levels of guthion in body tissues and fluids for populations living near hazardous waste sites has been identified. This information is necessary to establish a database that can be used to assess the need to conduct follow-up human health studies of adult and children populations exposed to guthion.

One effort is now under way at ATSDR that will examine the extant data at the five NPL sites at which guthion has been found. When complete, this database will include maximum concentrations of guthion in on-site and off-site media, and an indication of relevant routes of exposure. This database will be developed and evaluated before the need to collect additional media-specific data is assigned priority. This database will not, however, supply information on the levels of guthion (or its metabolites) in the tissues of adults and children living near hazardous waste sites or other exposed populations such as workers.

Although there is a need to collect data on levels of guthion in body tissues and fluids for populations living near hazardous waste sites, it is not considered a priority at this time because guthion has a short biological half-life and current analytical methods are not sufficient to determine short term guthion exposure in humans.

Thus, on the basis of the findings given in Section II and above, ATSDR is recommending the initiation of research or studies to fill the following exposure data needs (Table 3):

- None of the identified exposure data needs are considered to be priority at this time.

B. Toxicity

The available inhalation, oral, and dermal exposure studies indicate that the nervous system is the target organ of toxicity for guthion. Additional developmental toxicity studies via oral exposure are considered priority. It has been shown that acetylcholine, acetylcholinesterase, and butyrylcholinesterase are involved in the development of the nervous system and some of this development is not completed until adulthood. Given that acetylcholinesterase activity is highly sensitive to guthion, it is plausible that by interfering with the normal function and levels of these neurotransmitter and enzymes at critical periods during development, guthion might elicit adverse developmental effects in the nervous system. Thus, additional studies are needed to conduct a comprehensive evaluation of the neurodevelopmental effects guthion.

This nonhuman research need is justified because of the widespread domestic and environmental contamination of guthion, and the possibility that significant past exposures have affected many people.

Thus, on the basis of the findings given in Section II and above, ATSDR recommends the initiation of research or studies to fill the following toxicity priority data need (Table 3):

- Studies of developmental toxicity via oral exposure with emphasis on neurodevelopmental toxicity.

V. References

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Table 1. Exposure Data Needs

Exposure	Level I	Level II	Level III
Analytical	Methods for parent compound in REM*	Methods for degradation products in REM*	
	Methods for parent compound in blood or urine	Methods for parent compound/metabolites/biomarkers	
	Structure-activity relationships (SAR)		
Physical chemical properties	Water solubility		
	Volatility/vapor pressure		
	K_{ow}		
	Henry's law		Registries of exposed persons
Exposure levels	Production volume	Monitoring in REM*	Human dosimetry studies
	Use] may be used in lieu of monitoring data	Epidemiology
	Release/disposal		Monitoring for human exposure (personal sampling, biomarkers of exposure, tissue levels)
Environmental fate	Aerobic/anaerobic Biodegradation in H ₂ O	Exposures of children	
	Oxidation	Small field plot studies	
	Hydrolysis		
	Aerosolization	Monitoring for products in REM*	
	Photoreactivity		
	Volatilization		
	Soil adsorption/desorption		
Bioavailability		Food chain bioaccumulation	
		Availability from REM* (analytical or toxicity) emphasize <i>in vivo</i>	

*REM = Relevant Environmental Media

Table 2. Toxicity Data Needs

Toxicity	Level I	Level II	Level III
Single dose exposure	Single dose disposition Skin/eye irritation Acute toxicity		
Repeated dose exposure	14-day by relevant route 90-day subchronic	Comparative toxicokinetics*	
Chronic exposure	Structure-activity relationships (SAR)	1-Year chronic 2-Year bioassay	Epidemiology*
Genotoxicity*	Ames Micronucleus	Additional genotoxicity studies*	Mechanism of toxic action*
Endocrine disruption	<i>In vivo</i> & <i>in vitro</i> screen	2-Generation reproductive study	
Reproductive toxicity	Extended repro workup in subchronic	2-Generation or continuous breeding	Biomarkers*
			Clinical methods for mitigating toxicity*
Developmental toxicity*	Short term <i>in vivo</i> screen*	2-Species developmental*	Children's susceptibility**
Immunotoxicity	Use subchronic results	Immunotox battery	
Neurotoxicity	Neuropath in subchronic	Neurotox battery	
Sensitization	Dermal sensitization		
Carcinogenicity	Use muta & subchronic results	2-Year bioassay	

*Useful data for examining children's susceptibility issues

**Data needed for addressing children's susceptibility issues include genotoxicity (Level II), developmental toxicity (Levels I and II), epidemiology, mechanism of toxic action, biomarkers, and clinical methods for mitigating toxicity (Level III)

Table 3. ATSDR Substance-Specific Applied Research Program for Guthion

	EXPOSURE		
	Level I	Level II	Level III
Analytical	develop analytical methods for guthion in biological matrices		
Physical chemical properties			
Exposure levels		exp levels in environmental media	potential candidate for exposure registry
		exp levels in humans	
		exp levels in children	
Environmental fate			
Bioavailability		bioavailability of guthion from soil	
	TOXICITY		
	Level I	Level II	Level III
Acute	inhal, oral, dermal		
Repeated	inhal, oral, dermal		
Chronic		toxicokinetics	
Genotoxicity		inhal, oral, dermal	biomarkers
		<i>in vivo</i>	
Endocrine disruption	<i>in vitro</i> and <i>in vivo</i> screen		
Reproductive toxicity		Inhal, oral, dermal	
		inhal, oral, dermal	
Developmental toxicity		inhal, *ORAL* (neurodevelopmental), dermal	
Children's susceptibility			inhal, oral, dermal
Immunotoxicity		inhal, oral, dermal	
Neurotoxicity		inhal, oral, dermal	epidem
Sensitization			
Carcinogenicity		inhal, oral, dermal	

UPPER CASE: Priority Data Needs identified for guthion