

2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO GUTHION IN THE UNITED STATES

Guthion, a trade name for azinphos-methyl, is a restricted use organophosphate insecticide that is primarily used as a foliar application against phytophagous insect pests on fruit, field, or vegetable crops and works as both a contact insecticide and a stomach poison. In 2002, the estimated annual agricultural use of guthion had declined to about 1.2 million pounds, nearly a 50% decline from 1997 usage data. The total amount of guthion reportedly used in 1997 was 2,091,014 pounds, which was an 18% decrease from the amount used (2,548,867 pounds) in 1992. In the Interim Registration Eligibility Decision (interim RED) document for guthion, EPA estimated that <2 million pounds are used annually. The greatest amounts of guthion have historically been applied to orchard fruits such as apples, pears, cherries, and peaches; however, guthion has also been used extensively on cotton, almonds, sugarcane, and several other crops. The uses of guthion have been severely restricted in recent years. In 2001, the EPA proposed the immediate cancellation of most uses of guthion. Currently, the only crops that guthion can still be applied to are: almonds; apples/crabapples; blueberries, lowbush and highbush; Brussels sprouts; cherries, sweet and tart; nursery stock (woody shrubs, vines, seeding trees, and nonbearing fruit trees); parsley; pears; pistachios; and walnuts. On June 9, 2006, EPA proposed the cancellation of guthion for apples, blueberries, cherries, parsley, and pears by 2010 and a phase out of guthion's other uses by the end of 2007. On August 8, 2007, the companies that produce guthion advised the EPA that they intended to amend their registrations to terminate certain uses (Brussels sprouts and nursery stock) of guthion by September 30, 2007. Uses on walnuts, almonds, and pistachios are scheduled to cease by October 30, 2009, and all other uses would be terminated by September 30, 2012.

Guthion is not considered highly persistent in the environment and it degrades through 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. Background environmental levels of guthion are typically below analytical detection limits, and it is rarely detected in areas where it is not being used. Elevated levels of guthion are often detected during its application. For example, during the application of insecticides to an apple orchard in Massachusetts approximately 1 acre in size by airblast ground sprayers, guthion applied at 0.75 kg/ha was detected downwind of the spray zone (75 feet away) at a maximum concentration of 3.87 $\mu\text{g}/\text{m}^3$. Within 2 hours, the atmospheric level had dropped to 0.031 $\mu\text{g}/\text{m}^3$. Guthion has moderate to low mobility in soils based on K_{oc} values in the range of 475–3,266. Its leaching potential is considered low and is therefore only occasionally detected in groundwater.

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Guthion was only detected in 4 out of 2,451 groundwater samples collected from 1992 to 1996 in 20 major hydrological basins across the United States. Guthion is rarely detected in drinking water. In an analysis of finished drinking water in 12 states, guthion was detected in 5 out of 225 samples at a mean concentration of 0.059 µg/L and a maximum concentration of 0.114 µg/L. Spray drift following aerial application, as well as runoff and erosion of treated soils, often leads to contamination of rivers, lakes, ponds, and streams adjacent to fields or orchards where guthion has been used as an insecticide. Guthion was detected in 64 out of 98 surface water samples (maximum concentration 0.523 µg/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. More recent monitoring data from April to October 2004 from two sites near the Yakima River had guthion levels ranging from 0.013 to 0.042 µg/L. The frequency of detection for guthion at these two sampling locations were approximately 9 and 13%.

The most important route of exposure to guthion for the general population is through the ingestion of foods, especially fruits and vegetables 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. 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 (more recent surveys are not available). The dietary AVDI of guthion ranges from about 4 to 31 ng/kg/day (see Table 6-9). Agricultural workers, their family members including children, and persons residing near crops that are treated with guthion are expected to be exposed to higher levels than the general population. Since guthion is absorbed through the skin, dermal exposure to pesticide applicators or workers involved in picking, harvesting, and trimming of crops treated with guthion may be high. Although guthion is not considered highly volatile, dust samples in homes of agricultural workers, their vehicles, and personal items such as work clothing have been shown to contain detectable levels of guthion during the spraying season. This contaminated dust can be resuspended, resulting in dermal and inhalation exposures.

2.2 SUMMARY OF HEALTH EFFECTS

Available human and animal data suggest that reductions in acetylcholinesterase (AChE) activity are the most sensitive end points of guthion toxicity. In both humans and animals, erythrocyte AChE inhibition occurs at doses that are several times lower than doses eliciting clinical signs and symptoms. The neurotoxicity of guthion is dependent on its bioactivation via a cytochrome P450 mediated desulfuration to the oxon form gutoxon. Gutoxon inhibits the enzymatic action of nervous system AChE on the

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neurotransmitter acetylcholine, leading to the accumulation of acetylcholine at the ending of cholinergic nerves and resultant continual stimulation of electrical activity. Cholinergic nerves play an important role in the normal function of the neuromuscular, central nervous, endocrine, immunological, and respiratory systems. In this manner, exposure to guthion may lead to adverse effects on the normal function of many important systems.

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 AChE and plasma cholinesterase (ChE) activities. These studies reported no significant changes in plasma ChE or erythrocyte AChE activity in a small group of subjects ingesting 0.057–0.086 mg/kg/day for 4 weeks. Nevertheless, there is evidence suggesting that dermal, and perhaps inhalation, exposures of workers to guthion may lead to adverse health effects. An increased association was observed between the occurrence of systemic illness (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; however, interpretation of this study is complicated by the absence of worker exposure data and the potential exposure to other pesticides and formulation components. Although studies of agricultural workers have used the detection of urinary metabolites of guthion and ChE activity monitoring 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 AChE activity or whole blood ChE activity. These findings are in agreement with animal studies, which indicate that erythrocyte AChE 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 the lowest concentrations eliciting reductions in erythrocyte AChE activity. For instance, clinical signs, including hypercholinergy and nicotinic effects, salivation, lacrimation, exophthalmus, defecation, urination, and muscle fasciculations, have been observed in rats or mice administered single (16–26 mg/kg) or repeated (8 mg/kg/day) lethal oral doses of guthion and in rats and mice administered doses ≥ 3.2 mg/kg/day. However, doses in the range of 0.55–3 mg/kg/day in rats and dogs are sufficient to elicit 20–80% reductions in erythrocyte AChE activity with reductions $>80\%$ being observed at higher doses. Studies with rats and dogs suggest that reductions in erythrocyte AChE activity are not related to exposure duration. For instance, 75–92% reductions in erythrocyte AChE activity were observed in rats or dogs administered 2–4.3 mg/kg/day guthion on gestation days 6–15 or for 13 or 52 weeks and doses of 0.55–1.1 mg/kg/day elicited reductions in the 20–47% range in animals dosed for 13 weeks to 2 years.

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Erythrocyte AChE activity appears to be more sensitive than plasma ChE or brain AChE activity to the toxic effects of guthion. Biologically significant ($\geq 20\%$) reductions in erythrocyte AChE activity were observed in male and female rats exposed to 4.72 mg/m^3 guthion during 6 hours/day, 5 days/week for up to 12 weeks, but brain AChE activity was not affected and plasma ChE activity was reduced by $\geq 20\%$ only in females at one sampling time. Reductions in erythrocyte AChE activity have been observed in rats or dogs administered $\geq 0.55 \text{ mg/kg/day}$, whereas reductions in brain AChE and plasma ChE activity in rats and dogs were generally observed at $\geq 0.96 \text{ mg/kg/day}$.

Plasma ChE inhibition is used as an indicator of exposure, but does not serve as a reliable indicator of a neurotoxic effect. However, inhibition of erythrocyte and brain AChE represents a relevant neurological effect. Erythrocyte and/or brain AChE inhibition of 20–59% is considered to represent a less serious adverse neurological effect in the absence of more serious indicators of neurotoxicity. In this Toxicological Profile, “less serious” effects are those that are not expected to cause significant dysfunction or death, or those in which the significance to the organism is not entirely clear. The animal studies identified exposure levels at which guthion caused erythrocyte AChE inhibition in the absence of more serious indicators of neurotoxicity, which indicates that erythrocyte AChE inhibition at such exposure levels may represent the most sensitive effect for guthion toxicity.

No association was detected between occupational exposure to guthion and the occurrence of congenital malformations in a study of male agricultural workers in Spain during 1993 and 1994. Single oral doses $\geq 16 \text{ mg/kg}$ administered to mice during gestation, elicited reductions in fetal body weight and skeletal anomalies. Adverse developmental outcomes such as skeletal abnormalities, decreased pup weight and survival, reduced brain weight and ChE activity, and neuromuscular effects were observed in the offspring of pregnant rats or mice treated with $\geq 3.7 \text{ mg/kg/day}$ guthion during gestation and gestation and lactation. Developmental effects were not evident in rats or mice at oral doses $\leq 2.5 \text{ mg/kg/day}$. Reductions in litter and pup viability were observed in the fetuses of pregnant mice after a single oral dose of 20 mg/kg and in the offspring of rats after exposure to 1.3 mg/kg/day during gestation and lactation.

No studies were located regarding the carcinogenic potential of guthion in humans. A 2-year carcinogenicity study in rats showed an increased combined incidence of islet cell carcinoma or carcinomas of the pancreas in males receiving guthion in the diet for 80 weeks at a concentration resulting in an average dose of 10.9 mg/kg/day , followed by a 35-week observation period. However, this lesion occurs at a high spontaneous incidence in the animals used in this study and the increased incidence in the

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treated males could not be positively associated with guthion exposure. Similarly, increases in the incidence of benign thyroid tumors, malignant thyroid tumors, or combined follicular cell tumors observed in male rats exposed to 5.5 or 10.9 mg/kg/day could not be ascribed to treatment with guthion due to the high spontaneous incidence of these neoplasms in male rats in this laboratory. There was no evidence of the occurrence of treatment-related tumors in female rats in this study or in another study of male and female Wistar rats exposed to 0.25–3.11 mg/kg/day for 2 years. Benign and malignant neoplasms were observed among dosed and control B6C3F1 mice, but these lesions occur spontaneously in mice in this laboratory and the effect could not be positively associated with guthion exposure. The incidence of hepatocellular adenomas in male mice administered 5.4–10.7 mg/kg/day groups provide equivocal evidence of an association between these lesions and guthion exposure. There were no statistically significant associations between tumor incidence and guthion exposure in female mice. The results of these studies led the NCI to conclude that, under the conditions of this bioassay, guthion was not carcinogenic in male or female B6C3F1 mice or female Osborne-Mendel rats. The incidences of neoplasms of the pancreatic islets and of the follicular cells of the thyroid in male rats provide suggestive but insufficient evidence of the carcinogenic potential of guthion in male rats. The Department of Health and Human Services and the International Agency for Research on Cancer (IARC) 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. Currently, the EPA has no carcinogenicity classification for guthion.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for guthion. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an

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example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

Inhalation MRLs

- An MRL of 0.02 mg/m³ has been derived for acute-duration inhalation exposure (14 days or less) to guthion.

Only two studies examining the acute-duration toxicity of inhaled guthion were located. Kimmerle (1976) examined a number of end points in rats, but biologically significant alterations were limited to a 25% reduction in erythrocyte AChE activity in male rats exposed to 4.72 mg/m³, 6 hours/day, 5 days/week, for 2 weeks. The reduction in erythrocyte AChE activity in female rats was 18%. No adverse effects were observed in male or female rats at exposure concentrations ≤1.24 mg/m³. EPA (1978a) reported a 41% (range 27–59%) reduction in blood ChE activity in rats exposed to guthion aerosols (39 mg/m³) for 1 hour. The results of these studies are strongly supported by several acute- (Astroff and Young 1998; Pasquet et al. 1976), intermediate- (Allen et al. 1990; Holzum 1990; Sheets et al. 1997), and chronic-duration (Allen et al. 1990; Schmidt and Chevalier 1984) studies in rats and dogs, which identified reductions in erythrocyte AChE activity as the most sensitive end point following oral exposure to guthion. It is unclear if EPA (1978a) measured reductions in activity of whole blood or plasma ChE or erythrocyte AChE. Thus, the erythrocyte AChE inhibition observed in the Kimmerle (1976) study was selected as the basis of the acute-duration inhalation MRL.

In the study by Kimmerle (1976), SPF Wistar rats (10/sex/group) were exposed to aerosolized guthion at 0.195, 1.24, or 4.72 mg/m³, 6 hours/day, 5 days/week, for up to 12 weeks. Erythrocyte AChE activity measurements were made every 2 weeks after dosing began. Guthion aerosols were generated by first dissolving technical-grade guthion in a 1:1 solution of ethanol/polypropylene glycol. Ninety-seven percent of the droplets had a diameter of 1±0.5 µm (Kimmerle 1976). The animals were inspected daily and weighed weekly. Erythrocyte AChE and plasma ChE activities were determined after 2, 4, 6, 8, 10, and 12 weeks. There were no significant changes in appearance or behavior of male or female rats. After 2 weeks of exposure at 4.72 mg/m³, erythrocyte AChE activity was reduced by 25 and 18% in males and females, respectively. This study identified a no-observed-adverse-effect level (NOAEL) of 1.24 mg/m³ and a lowest-observed-adverse-effect level (LOAEL) of 4.72 mg/m³ for reductions in erythrocyte AChE activity in male rats.

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A NOAEL/LOAEL approach was used to derive a point of departure to estimate an acute-duration inhalation MRL for guthion. The lack of individual animal data or standard errors or standard deviations for the mean erythrocyte AChE activity precludes using a benchmark dose analysis approach. The NOAEL of 1.24 mg/m³ was adjusted for intermittent exposure (NOAEL_[ADJ]) and a human equivalent concentration (NOAEL_[HEC]) was calculated using the following equations:

$$\text{NOAEL}_{[\text{ADJ}]} = 1.24 \text{ mg/m}^3 \times 6 \text{ hours}/24 \text{ hours} = 0.31 \text{ mg/m}^3$$

$$\text{NOAEL}_{[\text{HEC}]} = \text{NOAEL}_{[\text{ADJ}]} \times \text{RDDR}_{\text{ER}} = 0.31 \text{ mg/m}^3 \times 1.626 = 0.50 \text{ mg/m}^3$$

The Regional Deposited Dose Ratio (RDDR) for the extrapulmonary (ER) effects was used to extrapolate deposited doses from rats to humans. The RDDR was calculated using EPA software (version 2.3) (EPA 1994b) with the following parameters: a particle size (mass median aerodynamic diameter, MMAD) of 0.88 μm with a default geometric standard deviation (sigma g) of 1.0, a default human body weight of 70 kg and minute volume of 13.8 L, and a rat body weight of 182 g (estimated from the data from Kimmerle 1976) and minute volume of 139 mL.

Based on the information provided by Kimmerle (1976) it was assumed that the sizes of the aerosol particles were log-normally distributed in a manner such that 1.5% of these were <0.5 μm and 1.5% were >1.5 μm. Based on these assumptions a geometric mean and geometric standard deviation of 0.9 and 0.23 μm, respectively, were calculated. These values were used to calculate a MMAD of 0.88 μm using the recommended equation in Table H-2 of the guidance document Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (EPA 1994b).

The NOAEL_[HEC] of 0.50 mg/m³ was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans using dosimetric adjustment and 10 for human variability), resulting in an acute-duration inhalation MRL of 0.02 mg/m³.

- An MRL of 0.01 mg/m³ was derived for intermediate-duration inhalation exposure (15–364 days) to guthion.

Only one study examining the intermediate-duration toxicity of inhaled guthion was located. Kimmerle (1976) examined a number of end points in rats, but biologically significant alterations were limited to a 26–48% reduction in erythrocyte AChE activity in male and female rats exposed to 4.72 mg/m³,

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6 hours/day, 5 days/week, for up to 12 weeks and a 20% reduction in body weight gain in male rats exposed to 4.72 mg/m³ for 12 weeks; no adverse effects were observed in rats at exposure concentrations ≤1.24 mg/m³. Support to the findings of Kimmerle (1976) is provided by several intermediate- (Allen et al. 1990; Holzum 1990; Sheets et al. 1997) and chronic-duration (Allen et al. 1990; Schmidt and Chevalier 1984) studies in rats and dogs, which identified a reduction in erythrocyte AChE activity as the most sensitive end point following oral exposure to guthion. Thus, the erythrocyte AChE inhibition observed in the Kimmerle (1976) study was selected as the basis of the intermediate-duration inhalation MRL.

In the study by Kimmerle (1976), SPF Wistar rats (10/sex/group) were exposed to aerosolized guthion at 0.195, 1.24, or 4.72 mg/m³, 6 hours/day, 5 days/week, for up to 12 weeks. Erythrocyte AChE activity measurements were made every 2 weeks after exposures began. Guthion aerosols were generated by first dissolving technical-grade guthion in a 1:1 solution of ethanol/polypropylene glycol. Ninety-seven percent of the droplets had a diameter of 1±0.5 µm (Kimmerle 1976). The animals were inspected daily and weighed weekly. Erythrocyte AChE and plasma ChE activities were determined after 2, 4, 6, 8, 10, and 12 weeks and determinations of hematology, serum glutamic-oxalacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), alkaline phosphatase (AP), urea, creatinine, and bilirubin were conducted after 12 weeks of exposure. At study termination, animals were sacrificed for gross examination. The thyroid, thymus, heart, lungs, liver, spleen, kidneys, adrenals, and gonads were weighed and examined histologically. Brain AChE activity was also determined. There were no significant changes in appearance or behavior of male or female rats. Male rats in the 4.72 mg/m³ group showed a 20% reduction in body weight gain after 12 weeks of exposure. No effects were detected in examinations of hemoglobin, erythrocyte counts, thrombocytes, packed cell volume, or leucocyte differential counts in male or female rats (Kimmerle 1976). There were also no effects on SGOT, SGPT, AP, urea, creatinine, or bilirubin. There were no observed differences in absolute or relative organ weights. There were no evident morphological changes or variations in organs or tissues in any of the rats. After 4–12 weeks of exposure at 4.72 mg/m³, erythrocyte AChE activity was reduced by 29–48 and 26–39% in males and females, respectively. The magnitude of the alterations in erythrocyte AChE activity established during the intermediate-duration time points did not appear to be exposure duration-related. No biologically significant alterations in erythrocyte AChE activity were observed at lower concentrations. The investigators noted that brain AChE activity was not reduced at any of the concentrations tested, but the brain AChE activity data were not provided. This study identified a NOAEL of 1.24 mg/m³ and a LOAEL of 4.72 mg/m³ for reductions in erythrocyte AChE activity in male rats.

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A NOAEL/LOAEL approach was used to derive a point of departure to estimate an intermediate-duration inhalation MRL for guthion. The lack of individual animal data or standard errors or standard deviations for the mean erythrocyte AChE activity precludes using a benchmark dose analysis approach. The NOAEL of 1.24 mg/m³ was adjusted for intermittent exposure (NOAEL_[ADJ]) and a human equivalent concentration (NOAEL_[HEC]) was calculated using the following equations:

$$\text{NOAEL}_{[\text{ADJ}]} = 1.24 \text{ mg/m}^3 \times 6 \text{ hours/24 hours} \times 5 \text{ days/7 days} = 0.22 \text{ mg/m}^3$$

$$\text{NOAEL}_{[\text{HEC}]} = \text{NOAEL}_{[\text{ADJ}]} \times \text{RDDR}_{\text{ER}} = 0.22 \text{ mg/m}^3 \times 1.695 = 0.37 \text{ mg/m}^3$$

The RDDR for the extrapulmonary (ER) effects was used to extrapolate deposited doses from rats to humans. The RDDR was calculated using EPA software (version 2.3) (EPA 1994b) with the following parameters: a particle size MMAD of 0.88 µm with a default geometric standard deviation (sigma g) of 1.0, a default human body weight of 70 kg and minute volume of 13.8 L, and a rat body weight of 253 g (from Kimmerle 1976) and minute volume of 182 mL.

Based on the information provided by Kimmerle (1976), it was assumed that the sizes of the aerosol particles were log-normally distributed in a manner such that 1.5% of these were <0.5 µm and 1.5% were >1.5 µm. Based on these assumptions, a geometric mean and geometric standard deviation of 0.9 and 0.23 µm, respectively, were calculated. These values were used to calculate a MMAD of 0.88 µm using the recommended equation in Table H-2 of the guidance document Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (EPA 1994b).

The NOAEL_[HEC] of 0.37 mg/m³ was divided by an uncertainty factor of 30 (3 for extrapolation from animals to humans using dosimetric adjustment and 10 for human variability), resulting in an intermediate-duration inhalation MRL of 0.01 mg/m³.

- An MRL of 0.01 mg/m³ was derived for chronic-duration inhalation exposure (365 days or more) to guthion.

No studies were located that allowed the derivation of a chronic-duration inhalation MRL. However, the available acute- and intermediate-duration inhalation studies and the acute-, intermediate-, and chronic-duration oral exposure studies support adopting the intermediate-duration MRL for chronic-duration exposures.

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Erythrocyte AChE activity was reduced by 29–48% in male rats and 26–39% in female rats exposed to guthion aerosols at 4.72 mg/m³ for 4–12 weeks without evident biologically significant changes in activity within the observation period (Kimmerle 1976). Similarly, intermediate- and chronic-duration oral exposures to 0.69–0.78 mg/kg/day in dogs (Allen et al. 1990) and 0.75–0.96 mg/kg/day in rats (Schmidt and Chevalier 1984) demonstrated biologically significant reductions in erythrocyte AChE activity that did not increase in severity with increasing exposure duration for up to 2 years (Allen et al. 1990; Schmidt and Chevalier 1984). Thus, a chronic-duration inhalation MRL of 0.01 mg/m³ is adopted from the intermediate-duration inhalation MRL and supported by the intermediate- and chronic-duration oral exposure studies in dogs and rats, which suggest that there are no duration-dependent increases in the severity of the inhibition of erythrocyte AChE activity.

Oral MRLs

- An MRL of 0.01 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to guthion.

Dose-related reductions of 23–82% in erythrocyte AChE activity were observed in rats after single oral doses of 2–18 mg/kg guthion (Pasquet et al. 1976) and in female rats following daily oral doses of 2 mg/kg/day on gestation days 6–15 (Astroff and Young 1998). Dose-related reductions of 21–75% of brain AChE activity were observed in rats after single oral doses of 2–18 mg/kg/day (Pasquet et al. 1976). Although reductions >20% were observed at the lowest dose 2 hours after exposure, brain AChE activity levels had returned to 94–95% of control values after 5 and 24 hours (Pasquet et al. 1976). Brain AChE activity was reduced by 27–40% in rats after repeated oral doses of 2 mg/kg/day on gestation days 6–15 (Astroff and Young 1998) and by 78% in female rats administered 5.7 mg/kg/day guthion in the feed for 1 week (Su et al. 1971). Su et al. (1971) estimated that 4.0 mg/kg/day of guthion were required to reduce brain AChE activity by 50%. Clinical signs of neurotoxicity such as lacrimation, salivation, defecation, and muscle fasciculations were observed in rats and mice receiving lethal oral doses ≥ 8 mg/kg/day (EPA 1978a; Short et al. 1980). Developmental effects such as increased incidence of supernumerary ribs and malaligned sternbrae, and reduced fetal weight and viability of litters were observed in mice or rats at doses ≥ 5 mg/kg (Kavlock et al. 1985; Pasquet et al. 1976; Short et al. 1980). The available data suggest that reduction in erythrocyte AChE activity is the most sensitive end point following acute-duration oral exposures to guthion. Although the studies by Astroff and Young (1998) and Pasquet et al. (1976) identified a LOAEL of 2 mg/kg/day for significant reductions in erythrocyte

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AChE activity, only the study by Astroff and Young (1998) identified a NOAEL (1 mg/kg/day) and thus, it was selected for derivation of the acute-duration oral MRL.

Pregnant Sprague-Dawley rats were administered guthion (87.7% active ingredient [a.i.]) at 0.5, 1.0, or 2.0 mg/kg/day by gavage on gestation days 6–15. Erythrocyte AChE was determined on gestation days 16 and 20 and brain AChE activity was determined on day 20 (Astroff and Young 1998). Inseminated females were examined daily for clinical signs. Dam body weight was determined on gestation days 0, 6, 8, 10, 12, 15, and 20. Food consumption was also determined periodically. Two groups of dams were used to establish maternal plasma ChE and erythrocyte and brain AChE activity on gestation days 16 and 20. Gross pathological examination of dams was conducted. Several reproductive and developmental end points, including early or late resorptions, implantation losses, and fetal survival, growth, and malformations, were evaluated. The reduction in AChE activity was the most sensitive end point in this study.

A >80% reduction in erythrocyte AChE activity was observed 24 hours after the last 2 mg/kg/day dose. A 40% reduction in brain AChE activity was also observed in dams in the 2 mg/kg/day group. Maternal plasma ChE activity in the 2.0 mg/kg/day group was approximately 30% lower than in controls on gestation day 16, but the effect was not statistically significant. On gestation day 20, maternal brain AChE activity remained 27% lower than control values but erythrocyte AChE and plasma ChE activities were not significantly different from those in control animals (Astroff and Young 1998). In spite of the magnitude of the AChE and ChE activity reductions, no adverse clinical signs were observed in the treated dams. There were no reductions in brain or erythrocyte AChE or plasma ChE activities in rats administered 0.5 or 1 mg/kg/day (Astroff and Young 1998).

In order to derive a point of departure to calculate an acute-duration oral MRL, a benchmark dose (BMD) approach was applied to the changes in erythrocyte AChE activity observed in female rats exposed to guthion by gavage during gestation (Astroff and Young 1998). BMDs and the lower bound of the 95% confidence limits of the benchmark doses (BMDLs) were calculated using the EPA Benchmark Dose Software (BMDS version 1.3.2; available from EPA). The BMDs and BMDLs are estimates of the doses associated with a 20% change in erythrocyte AChE activity. Reductions in erythrocyte AChE activity of magnitude <20% are not considered to be biologically significant. The BMD modeling is described in greater detail in Appendix A. The BMD and BMDL predicted from the power model are 1.33 and 1.04 mg/kg/day, respectively.

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An acute-duration oral MRL of 0.01 mg/kg/day was calculated by dividing the BMDL of 1.04 mg/kg/day by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

- An MRL of 0.003 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to guthion.

Dose-related reductions of 37–84% in erythrocyte AChE activity were observed in rats receiving 0.91–3.2 mg/kg/day guthion from the feed for 13 weeks (Sheets et al. 1997) and in male and female rats given guthion in the feed at 1.3 and 0.55 mg/kg/day, respectively, for 14 weeks and during mating, gestation, and parturition (Holzum 1990). Erythrocyte AChE activity was inhibited by 22–40 and 66–88% in male dogs receiving 0.69 and 3.8 mg/kg/day, respectively, from the feed for up to 26 weeks and reductions of 20–43 and 86–92% were observed in female dogs receiving 0.78 and 4.3 mg/kg/day, respectively, for 26 weeks (Allen et al. 1990). Clinical signs such as tremors, salivation, uncoordinated gait, and diarrhea were observed in rats administered guthion in the feed at ≥ 3.2 mg/kg/day guthion for 13 weeks (Sheets et al. 1997), in rats administered 5 mg/kg/day during gestation (Short et al. 1980), or in dogs receiving ≥ 0.69 mg/kg/day for 26 weeks (Allen et al. 1990). The available data suggest that reduction in erythrocyte AChE activity is the most sensitive end point following intermediate-duration oral exposures to guthion. The studies by Allen et al. (1990) and Sheets et al. (1997) identified LOAELs of 0.69–0.78 mg/kg/day in dogs and 0.91–1.1 mg/kg/day in rats, respectively, for reductions in erythrocyte AChE activity; however, the study by Allen et al. (1990) showed that dogs were more sensitive than rats to the anticholinesterase effects of guthion and thus, it was selected for derivation of the intermediate-duration oral MRL.

Technical-grade guthion (91.9% a.i.) was given to beagle dogs (4/sex/group) in the food at concentrations of 5.0, 25.0, or 125.0 ppm; these concentrations are equivalent to guthion doses of 0.15, 0.69, and 3.8 mg/kg/day, respectively, in the males, and 0.16, 0.78, and 4.3 mg/kg/day, respectively, in the females (Allen et al. 1990). Dose-related reductions in erythrocyte AChE activity were evident at the week 4 sampling time. Erythrocyte AChE activity was further reduced from week 4 to 13, but remained relatively constant from week 13 to week 26. Statistically nonsignificant reductions in erythrocyte AChE activity during the 26-week period were $\leq 8\%$ in males at 0.15 mg/kg/day and 11–21% in females at 0.16 mg/kg/day. Reductions in erythrocyte AChE activity were 22–40% in males at 0.69 mg/kg/day and 20–43% in females at 0.78 mg/kg/day. Reductions in erythrocyte AChE activity from weeks 4 to 26 were 66–88% in males (3.8 mg/kg/day) and 86–92% in females (4.3 mg/kg/day). Male and female dogs receiving guthion doses of 3.8 and 4.3 mg/kg/day, respectively, suffered from an increased incidence of

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mucoid diarrhea and occasional emesis. The same signs, but with a greater severity, were observed in male dogs at 0.69 mg/kg/day. These signs were related to treatment with guthion. Terminal body weights were reduced by 12 and 16% in male and female dogs of the 3.8 and 4.3 mg/kg/day dose groups, respectively, although there was no difference in food consumption among treated and control animals. There were no treatment-related hematological effects or changes in urinalysis parameters. Findings were negative in hearing and ophthalmoscopic tests on weeks 13 and 26 and there was no treatment-related increase in mortality in any dose group (Allen et al. 1990). Clinical chemistry tests showed that albumin and albumin/globulin values were significantly reduced in males by 13 and 20%, respectively, in the 3.8 mg/kg/day group.

In order to derive a point of departure to calculate an intermediate-duration oral MRL, a BMD approach was applied to the changes in erythrocyte AChE activity observed in male and female dogs exposed to guthion in the diet for 26 weeks (Allen et al. 1990). BMDs and BMDLs were calculated using the EPA Benchmark Dose Software (BMDS version 1.3.2). The BMDs and BMDLs are estimates of the doses associated with a 20% change in erythrocyte AChE activity. Reductions in erythrocyte AChE activity of magnitude <20% are not considered to be biologically significant. The BMD modeling is described in greater detail in Appendix A. A nonhomogeneous variance linear model predicted a BMD and BMDL of 0.44 and 0.29 mg/kg/day, respectively.

An intermediate-duration oral MRL of 0.003 mg/kg/day was calculated by dividing the BMDL of 0.29 mg/kg/day by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

- An MRL of 0.003 mg/kg/day has been derived for chronic-duration oral exposure (365 days or more) to guthion.

Dose-related reductions in erythrocyte AChE activity were observed in rats and dogs administered guthion in the feed for chronic durations. Statistically significant reductions in erythrocyte AChE activity were observed in male and female rats receiving 0.75 and 0.96 mg/kg/day guthion, respectively, from the feed for 2 years; however, these were only 10–22% reductions. Biologically significant reductions were mainly restricted to doses of 2.33 and 3.11 mg/kg/day in male (20–37% reduction) and female (23–31% reduction) rats, respectively (Schmidt and Chevalier 1984). Dogs appeared to be more sensitive to guthion. After 1 year, male dogs receiving 0.69 or 3.8 mg/kg/day showed reductions in erythrocyte AChE activity of 27 and 86%, respectively, while females receiving 0.78 or 4.3 mg/kg showed reductions in erythrocyte AChE activity of 35 and 86%, respectively (Allen et al. 1990). Significant reductions (19–

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67%) in plasma ChE activity were observed in female rats at doses ≥ 0.96 mg/kg/day and in male rats at 2.3 mg/kg/day (Schmidt and Chevalier 1984). In male dogs fed guthion for 1 year, reductions in plasma ChE activity were 53% at 3.8 mg/kg/day while female dogs showed reductions of 30 and 53% at 0.78 and 4.3 mg/kg/day, respectively (Allen et al. 1990). Plasma ChE inhibition is used as an indicator of exposure, but does not serve as a reliable indicator of a neurotoxic effect. However, inhibition of red blood cell AChE and brain AChE represents a relevant neurological effect. Increased relative brain and liver weights, lower terminal body weight, and alopecia were observed in rats at 2.3 mg/kg/day (Schmidt and Chevalier 1984). Increased incidence of diarrhea and occasional emesis were observed in male dogs at 0.69 mg/kg/day. Diarrhea, occasional emesis, and reductions in terminal body weight were also observed in male and female dogs receiving guthion at 3.8 and 4.3 mg/kg/day, respectively, from the feed for 1 year (Allen et al. 1990). The available chronic-duration data indicate that reduction in erythrocyte AChE activity is the most sensitive end point following chronic-duration oral exposures to guthion. The 52-week study in dogs (Allen et al. 1990) was selected to derive the chronic-duration oral MRL because, at similar doses (0.69–0.78 mg/kg/day in dogs after 52 weeks and 0.75–0.96 mg/kg/day in rats after 2 years), there was a more marked reduction in erythrocyte AChE activity in dogs (20–43%) than in rats (10–22%).

Technical-grade guthion (91.9% a.i.) was administered in the feed at 5, 25, or 125 ppm to beagle dogs (4/sex/group) for 52 weeks (Allen et al. 1990). These dietary levels provided guthion doses of 0.15, 0.69, and 3.8 mg/kg/day, respectively, to the males, and 0.16, 0.78, 4.3 mg/kg/day, respectively, to the females. Erythrocyte AChE and plasma ChE activities were determined prior to treatment and periodically until study termination. Dose-related reductions in erythrocyte AChE activity were evident on week 52. A statistically nonsignificant reduction of 15% in erythrocyte AChE activity was observed in females at 0.16 mg/kg/day on week 52, but there was no effect in males. On week 52, reductions in erythrocyte AChE activity in males at 0.69 and 3.8 mg/kg/day were 27 and 86%, respectively. Females in the 0.78 and 4.3 mg/kg/day groups showed 35 and 86% reductions, respectively, in erythrocyte AChE activity. Brain AChE activity on week 52 in the 3.8 and 4.3 mg/kg/day groups was reduced by 27 and 20% in males and females, respectively. Reductions in brain AChE activity were 1 and 10% in female and male dogs receiving administered 0.78 and 0.69 mg/kg/day, respectively. No effect on brain AChE activity was observed in males administered 0.15 mg/kg/day or females administered 0.16 mg/kg/day. Plasma ChE activity was reduced by 53% in males and females administered 3.8 and 4.3 mg/kg/day, respectively. No statistically significant reductions in plasma ChE activity were observed in male or female dogs administered ≤ 0.69 or ≤ 0.78 mg/kg/day, respectively. Terminal body weights were reduced by 12% in males in the 3.8 mg/kg/day group and by 16% in females in the 4.3 mg/kg/day group, although

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there was no difference in food consumption among treated and control animals. There were no treatment-related hematological effects or changes in urinalysis parameters. Findings were negative in hearing and ophthalmoscopic tests conducted at study termination and there was no treatment-related increase in mortality in any dose group. There were no changes in absolute or relative organ weights in females at the doses tested. Absolute and relative spleen weights were reduced in males in a dose-related manner with significant reductions in relative spleen weight at doses ≥ 0.69 mg/kg/day; however, congestion of the spleen and increased absolute spleen weight were observed in 4/4 male dogs of the control group. A 7–17% decrease in albumin and albumin/globulin values were observed on week 52 in males of the 3.8 mg/kg/day group. A 39 and 15% increase in P450 activity was observed in male dogs at 3.8 mg/kg/day and in female dogs at 4.3 mg/kg/day, respectively. A 34 and 30% increase in N-demethylase activity was observed in male dogs at 3.8 mg/kg/day and in female dogs at 4.3 mg/kg/day, respectively. Other effects were restricted to the high dose groups (Allen et al. 1990).

In order to derive a point of departure to calculate a chronic-duration oral MRL, a BMD approach was applied to the changes in erythrocyte AChE activity observed in male and female dogs exposed to guthion in the diet for 52 weeks (Allen et al. 1990). BMDs and BMDLs were calculated using the EPA Benchmark Dose Software (BMDS version 1.3.2). The BMDs and BMDLs are estimates of the doses associated with a 20% change in erythrocyte AChE activity. Reductions in erythrocyte AChE activity of magnitude <20% are not considered to be biologically significant. The BMD modeling is described in greater detail in Appendix A. BMDs of 0.48 and 0.50 mg/kg/day in male and female dogs, respectively, and BMDLs of 0.30 and 0.32 mg/kg/day in male and female dogs, respectively, were obtained by analysis of the low-dose region of the dose-response curve for dogs exposed to guthion in the diet for 52 weeks. The lowest BMDL (0.30 mg/kg/day) was selected as the point of departure.

A chronic-duration oral MRL of 0.003 mg/kg/day was calculated by dividing the BMDL of 0.30 mg/kg/day by an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).