2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO 1,4-DIOXANE IN THE UNITED STATES

1,4-Dioxane is a stable, clear liquid at ambient temperatures and is miscible with water. It is used primarily as a solvent for chemical processing (e.g., adhesives, cleaning and detergent preparations, cosmetics, deodorant fumigants, emulsions and polishing compositions, fat, lacquers, pulping of wood, varnishes, waxes). It has also been used as a laboratory reagent; in plastic, rubber, insecticides, and herbicides; as a chemical intermediate; as part of a polymerization catalyst; and as an extraction medium of animal and vegetable oils. 1,4-Dioxane may also be found as a contaminant in ethoxylated surfactants, which are used in consumer cosmetics, detergents, and shampoos. Currently, manufacturers remove 1,4-dioxane from ethoxylated surfactants to low levels by vacuum stripping.

Human exposure to 1,4-dioxane may occur by inhalation, ingestion, and dermal contact. Because 1,4-dioxane may be found in tap water, human exposure to 1,4-dioxane may also occur during activities such as showering, bathing, and laundering. Exposure to 1,4-dioxane in tap water through inhalation during showering or other indoor activities can result in higher exposures to 1,4-dioxane compared to ingestion of drinking water.

Current levels of 1,4-dioxane in ambient air, drinking water, and food samples are not available. In the mid 1980s, levels of 1,4-dioxane in ambient outdoor air ranged from 0.1 to 0.4 $\mu g/m^3$ (0.028–0.11 ppb). Mean concentrations of 1,4-dioxane in indoor air were a factor of 10 higher at 3.704 $\mu g/m^3$ (1.029 ppb). In the 1970s, municipal water supplies in the United States were reported to contain 1 µg/L (ppb) of 1,4-dioxane. 1,4-Dioxane has been detected in food volatiles which may indicate that 1,4-dioxane may be a natural constituent in some foods. Volatiles from chicken, meat, tomatoes, and small shrimp have been reported to contain 1,4-dioxane at unquantified levels. Dermal exposure to 1,4-dioxane may occur with the use of consumer cosmetics, detergents, and shampoos containing ethoxylated surfactants. Between the years 1992 and 1997, the average concentration of 1,4-dioxane in cosmetic finished products was reported to fluctuate from 14 to 79 ppm (mg/kg). In a more recent survey reported by the Campaign for Safe Cosmetics, the levels of 1,4-dioxane in cosmetic products that were tested were found to be lower than in the survey done by the FDA in the 1990s. FDA issued a regulation in 1986 that requires labels on foaming detergent bath products to bear adequate directions for safe use and a caution to keep the product

out of the reach of children (FDA 1986). If the product is intended for children's use, the label may add a caution requiring adult supervision.

2.2 SUMMARY OF HEALTH EFFECTS

Limited information exists regarding the health effects of 1,4-dioxane in humans. Yet, the available data are sufficient to clearly identify the liver and kidneys as the target organs for 1,4-dioxane toxicity following short-term exposure to relatively high amounts of 1,4-dioxane, regardless of the route of exposure. This has been corroborated in studies in animals. Workplace exposures to undetermined, but presumably high concentrations of 1,4-dioxane have resulted in death. Inhalation was the most likely route of exposure, although considerable dermal contact may also have taken place in one of these cases. Evaluation of the subjects prior to death did not provide a picture that could be considered unique to 1,4-dioxane. Subjects often complained of gastrointestinal pain, had high blood pressure, anuria, and leukocytosis, and exhibited signs of nervous system involvement. The deaths occurred 5–8 days after the initial symptoms of illness. Postmortem evaluation revealed extensive liver and kidneys damage and in three out of five cases described in one study; kidney disease was considered to be the direct cause of death. Controlled exposures of volunteers to airborne 1,4-dioxane for periods ranging from a few minutes to 6 hours produced eye, nose, and throat irritation. The lowest exposure concentration that produced eye irritation was 50 ppm during a 6-hour exposure, but exposure in a much older study to 2,000 ppm for 3 minutes produced no complaints of eye or nasal discomfort. In a more recent study, exposure of volunteers to 20 ppm for 2 hours did not induce eye or respiratory irritation. Little is known about long-term exposure to lower concentrations of 1,4-dioxane. A study of workers exposed to 0.006–14.3 ppm 1,4-dioxane for an average of 25 years found no evidence of liver or kidney disease or any other clinical effects. An additional study that examined mortality rates among workers employed at a manufacturing and processing facility found no differences between observed and expected incidences of cancer. However, this study was limited in size and exposure duration. Although no information was available regarding reproductive, developmental, or immunological effects specific to 1,4-dioxane in humans, some occupational studies of workers exposed to 1,4-dioxane in combination with other solvents have reported elevated rates of spontaneous abortion, stillbirths, premature births, and low birth weights. These effects cannot be attributed solely to 1,4-dioxane.

As previously mentioned, the liver and kidneys are also targets of 1,4-dioxane toxicity in animals and this has been described following inhalation, oral, and dermal exposure. There are no studies of the effects of 1,4-dioxane on reproductive function or immunocompetence in animals, and only one study in rats

evaluated developmental end points following oral exposure during gestation. Slight fetotoxicity occurred at a dose level that also affected the mothers. Chronic administration of 1,4-dioxane in the drinking water produced liver cancer in rats, mice, and guinea pigs, and cancer of the nasal cavity in rats. However, a 2-year inhalation study in rats exposed to relatively low concentrations of 1,4-dioxane (111 ppm) provided no evidence of carcinogenicity or any other health effect. The mechanism of carcinogenicity of 1,4-dioxane has not been elucidated, but the lack of or weak genotoxicity of 1,4-dioxane, its strong promotion properties, and the extensive cytotoxicity observed in some studies at dose levels that induce tumors suggest that 1,4-dioxane may be acting through a non-genetic mode of action.

Only cancer (with emphasis on the liver) and kidney effects are discussed below since these are the effects of most concern should humans be accidentally acutely exposed to high amounts of 1,4-dioxane or populations be identified that are being exposed to low-level, long-term exposure to this chemical.

Liver and Cancer Effects. Liver effects have occurred in humans and animals exposed to 1,4-dioxane, and the data in animals suggest that they occur regardless of the route of exposure. An occupational study and a case report provided a detailed description of the liver pathology in subjects following exposure to 1,4-dioxane that resulted in deaths within 1–2 weeks after the exposure. Upon postmortem examination, enlarged and pale liver and centrilobular necrosis were commonly observed. None of the subjects showed jaundice before death. Neither workers exposed to lower concentrations of 1,4-dioxane for many years nor volunteers exposed for a single 6-hour period to 50 ppm 1,4-dioxane showed indications of liver alterations.

One study provided detailed descriptions of liver pathology in several animal species exposed intermittently to 1,4-dioxane by inhalation for a period of up to 13 weeks and also exposed orally and by dermal contact. Both lethal and non-lethal concentrations (1,000–10,000 ppm) caused degrees of degeneration that varied from cloudy swelling to large areas of complete necrosis. Similar effects were seen following oral and dermal exposure. Hepatocyte vacuolation and swelling were reported in mice and rats dosed with 1,4-dioxane in the drinking water for 2 or 13 weeks. Evidence of hepatic degenerative changes was seen in Sherman rats that died after 2–4 months of treatment in a 2-year drinking water bioassay. Long-term oral studies in animals described hepatocellular degeneration and necrosis in Sherman rats at about 94 mg 1,4-dioxane/kg/day and liver hyperplasia in Fischer 344 rats at about 81 mg/kg/day; hepatocytomegaly was observed in female Osborne-Mendel rats treated with

approximately 350 mg/kg/day. The apparent different lesions and thresholds for the effects in the liver may reflect strain differences.

The mechanism by which 1,4-dioxane induces liver damage in unknown. Results from some studies suggest that toxicity occurs at high doses when the metabolism of 1,4-dioxane is saturated, which would suggest that the parent compound is the toxic form. This also is consistent with more recent observations that induction of hepatic CYP2B1/2 and CYP2E1 did not play a role in the toxicity of 1,4-dioxane, which suggested that highly reactive and toxic intermediates do not play a major role in the liver toxicity of 1,4-dioxane, even under conditions of enhanced metabolism. On the other hand, it has also been reported that the metabolite, 1,4-dioxane-2-one, was several-fold more toxic than 1,4-dioxane based on intraperitoneal LD_{50} determinations in rats.

All long-term studies in rats dosed with 1,4-dioxane via the drinking water reported an increased incidence of liver tumors. In the better reported studies, tumor development occurred at doses that produced hepatocellular hyperplasia and degeneration and evidence of hepatic regeneration. 1,4-Dioxane also induced tumors in the nasal turbinates in rats and liver tumors in mice and guinea pigs. The mechanism of carcinogenicity of 1,4-dioxane has not been elucidated, but the results from several lines of investigation have led some to conclude that 1,4-dioxane has a non-genotoxic, yet unknown, mode of action.

The EPA has developed cancer risk values for 1,4-dioxane based on the increased incidence of tumors of the nasal cavity in male Osborne-Mendel rats in a 2-year drinking-water bioassay. The relevance of these tumors to humans has been questioned. It was suggested that the tumors resulted from inspiration of water containing 1,4-dioxane into the nasal cavity. Preliminary studies with a dye in the drinking water have demonstrated that large amounts of inhaled water may be deposited directly in the nose. The lack of nasal tumors in mice in chronic oral studies could be due to different tissue sensitivity and/or repair mechanism, or to a difference in anatomical features. Also, the lack of nasal cytotoxicity and nasal tumors in Wistar rats exposed intermittently to 111 ppm 1,4-dioxane in the air for 2 years suggests that the minimal effective dose may not have been reached.

Liver toxicity has been proposed to be necessary for liver tumor formation possibly in rats. Since this suggests to some scientists the existence of a threshold, they have suggested using approaches other than the Linearized Multistage Model for estimating human cancer risk due to exposure to 1,4-dioxane. In addition, the use of available physiologically based pharmacokinetic (PBPK) models may provide a

means for estimating the internal dose of 1,4-dioxane or metabolites delivered to the target organ from the doses administered in the animal bioassays. Based on inadequate evidence in humans and sufficient evidence in experimental animals, the International Agency for Research on Cancer (IARC) has determined that 1,4-dioxane is possibly carcinogenic to humans. The Department of Health and Human Services (DHHS) has stated that 1,4-dioxane is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity in experimental animals. The EPA has established that 1,4-dioxane is a probable human carcinogen based on inadequate evidence in humans and sufficient evidence in animals. The EPA is currently re-evaluating the health assessment for 1,4-dioxane.

Renal Effects. Kidney lesions appeared to be the cause of death of five workers who were exposed to unknown concentrations of 1,4-dioxane primarily by the inhalation route. Death occurred 1–2 weeks after episodes of elevated exposure started at work. All five cases experienced oliguria or anuria. Post mortem examination revealed swollen kidneys with hemorrhages and necrosis of the cortex. Similar findings were reported in a fatal case report. No renal alterations, as judged by urinalyses, were described in other reports of long-term occupational exposure to low levels of 1,4-dioxane or in a group of volunteers following a single 6-hour exposure to 50 ppm 1,4-dioxane. Very similar kidney lesions were observed in animals exposed to 1,4-dioxane by several routes of exposure. Rodents exposed to acutely lethal concentrations of 1,4-dioxane showed severe kidney damage consisting in marked patchy cell degeneration of the cortical tubules and intense vascular congestion and hemorrhages both inter- and intra-tubular. Well-marked kidney lesions were present in animals that survived intermittent inhalation exposure for up to 12 weeks. Similar observations were made in intermediate-duration studies in rats and mice exposed orally and in guinea pigs and rabbits following dermal application of 1,4-dioxane. Evidence of renal degenerative changes was seen in Sherman rats that died after 2–4 months of treatment in a 2-year drinking water bioassay. Nuclear enlargement of the proximal tubule was reported in rats in a 13-week study. Increased incidence of degeneration and necrosis of the tubular epithelium was seen in rats that survived until termination of the study and similar findings were reported in Osborne-Mendel rats. Hematuria and nuclear enlargement of the proximal tubule were reported in Fischer-344 rats, and hematuria, proteinuria, and glucosuria were noted in Crj:BDF_1 mice in a 2-year drinking water study. No compound-related neoplastic lesions were observed in the kidneys in other long-term studies conducted with 1,4-dioxane in rodents. The mechanism(s) by which 1,4-dioxane induces kidneys lesions is not known, and virtually no discussion about this topic was found in the reviews available. The findings in the case studies are consistent with an acute nephritic syndrome, which is characterized by acute renal failure and oliguria. It is not expected that exposure to concentrations commonly in the environment would cause adverse kidney effects in humans.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for 1,4-dioxane. 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 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 2 ppm has been derived for acute-duration inhalation exposure (14 days or less) to 1,4-dioxane.

The acute-duration inhalation MRL is based on a no-observed-adverse-effect level (NOAEL) of 20 ppm for eye and respiratory irritation and pulmonary function effects in humans (Ernstgård et al. 2006). In that study, six male and six female volunteers were exposed to 0 or 20 ppm 1,4-dioxane vapor for 2 hours under dynamic conditions. Each subject was exposed on two separate occasions to 0 or 20 ppm. End points monitored included self-rated symptoms on a visual analogue scale that measured discomfort of the eyes, nose and throat, breathing difficulty, solvent smell, headache, fatigue, nausea, dizziness and 'feeling of intoxication'. Rating was performed before, during (3, 60, and 118 minutes), and after exposure (20 and 180 minutes). Respiratory function was assessed by spirometry before exposure, immediately after and 3 hours after exposure ceased. The specific parameters measured included vital capacity, forced vital capacity, forced expiratory volume in 1 second, peak expiratory flow, and forced expiratory flow at 25, 50, and 75% of the force vital capacity. Also assessed was nasal swelling before, immediately after,

and 3 hours after exposure. Eye blinking was monitored throughout the exposure period by electromyography. Also, two inflammatory markers, high sensitivity C reactive protein and interleukin 6, were measured in blood before and 3 hours after exposure. Exposure to 1,4-dioxane under the conditions of the study did not significantly affect any of the end points monitored except the perception of smell of the chemical, which increased significantly after 3, 60, and 118 minutes if exposure. The NOAEL of 20 ppm was divided by an uncertainty factor of 10 (for human variability) to yield the MRL of 2 ppm. An adjustment to 24-hour exposure was not necessary because the first effects observed, as shown by Young et al. (1977, see below), are local irritation effects that are not time-dependent.

Support for the acute-duration inhalation MRL of 2 ppm is provided by a study by Young et al. (1977) in which four healthy male volunteers were exposed to 50 ppm 1,4-dioxane for 6 hours under dynamic airflow conditions. Prior to the study, the subjects provided a complete history and underwent tests including chest x-ray, EKG, respiratory function tests, a conventional battery of 12 blood chemistry tests plus triglyceride and creatinine determinations, and complete hematological and urine analyses. Except for the chest x-ray, the tests were repeated 24 hours and 2 weeks after the exposure. The tests conducted 24 hours and 2 weeks after exposure did not reveal any exposure-related abnormalities, although no data were provided in the study. Eye irritation was a frequent and the only complaint throughout the exposure. Tolerance to the odor of 1,4-dioxane occurred during exposure. Two of the subjects could not perceive the odor after 4 and 5 hours in the chamber. The 50 ppm exposure level constitutes a minimal LOAEL for eye irritation, although there was no control experiment, and possible low humidity in the exposure chamber (not addressed in the report) might have contributed to the eye irritation.

Other studies with volunteers also support the findings of Ernstgård et al. (2006) and Young et al. (1977). For example, Silverman et al. (1946) exposed 12 subjects to various concentrations of 1,4-dioxane for only 15 minutes and determined a no-observed-adverse-effect level (NOAEL) of 200 ppm for eye and nose irritation; the LOAEL was 300 ppm. Wirth and Klimmer (1936) reported that slight mucous membrane irritation started to take place in volunteers exposed to concentrations about 278 ppm for a few minutes (unspecified) and that at 1,390 ppm for several minutes, the subjects described prickling in the nose and scratchiness and dryness in the throat. Fairley et al. (1934) reported a NOAEL of 2,000 ppm (only level tested) for respiratory and ocular effects in six subjects exposed to 1,4-dioxane for only 3 minutes. Finally, Yant et al. (1930) described slight eye, nose, and throat irritation in a group of five subjects exposed to 1,600 ppm (only level tested) 1,4-dioxane for only 10 minutes. The available studies in animals used exposure concentrations that often caused death among the animals and were much higher than the concentrations tested by Ernstgård et al. (2006) and Young et al. (1977).

Because there were no adequate intermediate-duration inhalation studies in humans or animals from which to derive an intermediate-duration inhalation MRL, the chronic-duration inhalation MRL of 1 ppm (see derivation below) was adopted also for intermediate-duration exposure. The intermediate-duration database for 1,4-dioxane consists of one early study that reports the effects of 1,4-dioxane in several animal species exposed to high concentrations (lethal in some cases) of 1,4-dioxane (Fairley et al. 1934). Rats, mice, guinea pigs, and rabbits were exposed 3 hours/day, 5 days/week for periods of up to 12 weeks. At termination, examination of the animals revealed moderate to severe liver and kidney toxicity occurring at all exposure levels in all of the species tested. The lowest exposure level was 1,000 ppm.

- An MRL of 1 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to 1,4-dioxane.

The MRL is based on a NOAEL of 111 ppm for liver effects in Wistar rats (Torkelson et al. 1974) and application of the physiologically-based pharmacokinetic (PBPK) model of Reitz et al. (1990). Source code and parameter values for running the rat and human models in Advance Continuous Simulation Language (ACSL) were provided by Dr. Richard Reitz. A detailed description of the model and its application is presented in Appendix B. In the Torkelson et al. (1974) study, groups of Wistar rats (288/sex) were exposed to 1,4-dioxane vapors at a concentration of 0.4 mg/L (111 ppm) 7 hours/day, 5 days/week for 2 years. Controls were exposed to filtered room air. End points examined included clinical signs, eye and nasal irritation, skin condition, respiratory distress, and tumor formation. Hematological parameters (hemoglobin, red blood cell count, total and differential leukocyte counts, corpuscular volume) were determined after 16 and 23 months of exposure. Blood collected at termination was used also for determination of clinical chemistry parameters (serum alanine aminotransferase [ALT] and alkaline phosphatase activity, BUN, total protein). Liver, kidneys, and spleen were weighed and the major tissues and organs were processed for microscopic examination. Exposure to 1,4-dioxane vapors had no significant effect on mortality or body weight gain and induced no signs of eye or nasal irritation or respiratory distress. Slight but statistically significant changes in hematological and clinical chemistry parameters were within the normal physiological limits and were considered of no toxicological importance. Organ weights were not significantly affected. Microscopic examination of organs and tissues did not reveal treatment-related effects. Because the only exposure level tested did not cause any significant adverse effects, the true study NOAEL is likely to be higher than 111 ppm. Using the Reitz et al. (1990) model for interspecies extrapolation of 1,4-dioxane dosimetry for data from the Torkelson et al. (1974) study yields a human equivalent NOAEL of 35.5 ppm. Applying an uncertainty factor of 30 (3 for

using dosimetric adjustments and 10 for human variability) yields a chronic-duration inhalation MRL of 1 ppm. Using EPA's standard methodology for extrarespiratory effects for a category 3 gas rather than the PBPK model, and an uncertainty factor of 30, results in an MRL of 2 ppm for 1,4-dioxane. The derivation using the PBPK model is preferred because it yields a more protective MRL.

The limited human data supports the chronic-duration inhalation MRL. An occupational study by Thiess et al. (1976) provided no evidence of ill effects in a group of 74 German workers exposed to concentrations ranging from 0.006 to 14.3 ppm for an average of 25 years. In another epidemiological study, mortality rates were evaluated among workers exposed to 0.1–17 ppm 1,4-dioxane for up to 21 years (Buffler et al. 1978). No differences were found between observed and expected incidences of cancer.

Long-term oral studies in animals also support the liver as sensitive target for 1,4-dioxane toxicity. Liver hyperplasia, hepatocellular degeneration, and necrosis have been described in chronic-duration studies in rats (JBRC 1998c; Kociba et al. 1974; NCI 1978).

Oral MRLs

- An MRL of 4 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to 1,4-dioxane.

The acute-duration oral MRL is based on a NOAEL of 370 mg/kg/day for nasal effects in a study in rats (JBRC 1998a). In that study, F344/DuCrj rats (10/sex/group) were administered 1,4-dioxane in the drinking water in concentrations of 0, 1,110, 3,330, 10,000, 30,000, or 90,000 ppm for 2 weeks (0, 130, 370, 1,010, or 2,960 mg/kg/day for males; 0, 160, 400, 1,040, or 2,750 mg/kg/day for females). End points evaluated included clinical signs, food and water consumption, body weight, gross necropsy, and histopathology on 2–3 animals per group. All animals in the highest-dose groups died. Two females dosed with 2,750 mg/kg/day died. Body weight gain was reduced by about 25% in males dosed with 2,960 mg/kg/day and in females dosed with 2,750 mg/kg/day. Food and water consumption was reduced approximately by 30% in males and females dosed with 2,960 mg/kg/day and 2,750 mg/kg/day, respectively. Also in these groups, there was nuclear enlargement of the olfactory epithelium, swelling and vacuolar changes of the central area in the liver, hydropic change of the proximal renal tubule, and vacuolar changes in the brain. Nuclear enlargement of the olfactory epithelium occurred in males at 1,010 mg/kg/day (1/2 compared to 0/2 at 370 mg/kg/day) and in females at 1,040 mg/kg/day (2/2 compared to 0/2 at 400 mg/kg/day). The study NOAEL was 400 mg/kg/day in females and

370 mg/kg/day in males (3,330 ppm). Therefore, the dose level of 370 mg/kg/day in male rats is used as basis for the MRL. The MRL of 4 mg/kg/day was calculated by dividing the male NOAEL of 370 mg/kg/day by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability). It should be pointed out that the study has several limitations, including the lack of statistical analysis of the results, only a small number (2–3) of animals were examined, and end points such as hematology, clinical chemistry, clinical signs, and gross examinations were not conducted or reported. JBRC (1998a) conducted a similar study in male and female Crj:BDF_1 mice and identified NOAELs of 1,380 and 1,780 mg/kg/day for liver effects in males and females, respectively. Doses of 2,550 and 3,220 mg/kg/day caused swelling of the central area of the liver in males and females, respectively. No nasal effects were observed in the mice.

Most of the rest of the acute database consists of high-dose early studies aimed at determining LD_{50} values (de Navasquez 1935; Kesten et al. 1939; Laug et al. 1939; Pozzani et al. 1959; Smyth et al. 1941). The lowest dose that caused lethality was 327 mg 1,4-dioxane/kg/day in a study that tested only three dogs (Schrenk and Yant 1936). This dose was provided in the drinking water and killed one dog after 10 days of treatment. Doses of 375 mg/kg/day killed another dog in 9 days. However, because the dogs were allowed to drink the 1,4-dioxane solution only twice daily and no other source of water was available, dehydration may have played a role in the death of the animals. A gestational exposure study in rats identified a maternal and developmental NOAEL and LOAEL of 513 and 1,033 mg/kg/day, respectively (Giavini et al. 1985). Dams dosed with 1,033 mg/kg/day gained less weight than controls, and fetal weight in this group was reduced by 5.3% relative to controls. In addition, a slightly but significantly higher incidence of reduced sternum ossification was noticed in the high-dose group.

- An MRL of 0.6 mg/kg/day has been derived for intermediate-duration oral exposure (15–364 days) to 1,4-dioxane.

The intermediate-duration oral MRL is based on a NOAEL of 60 mg 1,4-dioxane/kg/day for liver effects in rats (JBRC 1998b). In that study, groups of F344/DuCrj rats (10/sex/group) were administered 1,4-dioxane in the drinking water in concentrations of 0, 640, 1,600, 4,000, 10,000, or 25,000 ppm for 13 weeks (0, 60, 150, 330, 760, or 1,900 mg/kg/day in males; 0, 100, 200, 430, 870, or 2,020 mg/kg/day in females). End points evaluated included clinical signs, food and water consumption, body weight, complete hematology and clinical chemistry tests, urinalysis, organ weights, gross necropsy, and histopathology. One female in the 2,020 mg/kg/day group died. Body weight gain was reduced at 870 mg/kg/day (12%) and 2,020 mg/kg/day (21%) in females and 1,900 mg/kg/day (21%) in males. Food consumption was reduced 13% in females at 2,020 mg/kg/day. Water consumption was reduced in a

dose-related manner in all male groups and in females at ≥200 mg/kg/day. Hematology tests showed significant increases in erythrocyte counts, hemoglobin, hematocrit, and neutrophils, and a decrease in lymphocytes in males at 1,900 mg/kg/day, and decreases in mean corpuscular volume and platelets in females at 2,020 mg/kg/day. Total protein and albumin were decreased in males at ≥330 mg/kg/day and in females at ≥430 mg/kg/day. Serum aspartate aminotransferase (AST), ALT, alkaline phosphatase (AP), and leucine aminopeptidase (LAP) activities, and levels of cholesterol, triglycerides, sodium, and glucose were significantly elevated in high dose males and females. Urinary pH was decreased in males at ≥330 mg/kg/day and in females at ≥870 mg/kg/day. Absolute and relative kidney weights were increased in females at ≥200 mg/kg/day. Nuclear enlargement of the respiratory epithelium occurred in males at ≥150 mg/kg/day and in females at ≥200 mg/kg/day; nuclear enlargement of the olfactory and tracheal epithelium occurred in males at ≥330 mg/kg/day and in females at ≥430 mg/kg/day. Swelling of the central area of the liver was observed in males at ≥150 mg/kg/day and in females at ≥870 mg/kg/day, and vacuolar changes in the liver occurred in males at ≥760 mg/kg/day and in females at 2,020 mg/kg/day. Nuclear enlargement of the proximal tubule of the kidneys was seen in males at ≥760 mg/kg/day and in females at ≥870 mg/kg/day. Hydropic changes in the proximal tubule of the kidneys and vacuolar changes in the brain occurred in high-dose males and females (1,900 and 2,020 mg/kg/day, respectively). The study LOAEL was 150 mg/kg/day for liver and nasal effects in male rats. Limitations of the study include the lack of reporting on clinical signs and gross necropsy. To derive the MRL, the NOAEL of 60 mg/kg/day for liver effects in males was divided by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability), yielding an intermediate-duration oral MRL of 0.6 mg/kg/day.

A study by Lundberg et al. (1987) supports the liver findings of JBRC (1998b). The study used male Sprague-Dawley rats (8–11/group) that were treated with 100 or 1,000 mg 1,4-dioxane/kg by gavage in saline 5 days/week for 7 weeks. One week after the last treatment, the rats were killed and the livers were processed for microscopic examination. The livers of high-dose rats showed enlarged foamy hepatocytes mainly in midzonal regions. The foamy appearance was due to vacuoles shown to contain fat. No treatment-related histopathological alterations were observed in the liver at the 100 mg/kg/day dose level. Also supporting the findings from JBRC (1998b) is a report by Stott et al. (1981) who found that repeated dosing of rats with 1,000 mg 1,4-dioxane/kg/day for 7 or 11 weeks produced hepatocyte swelling and histopathology. Similar findings were reported in an earlier study in which rats were treated with doses of approximately 1,428 mg 1,4-dioxane/kg/day in the drinking water for 34 days (Fairley et al. 1934).

- An MRL of 0.1 mg/kg/day has been derived for chronic-duration oral exposure (365 days or more) to 1,4-dioxane.

The chronic-duration oral MRL is based on a NOAEL of 9.6 mg 1,4-dioxane/kg/day for liver effects in male rats in a study by Kociba et al. (1974). In that study, groups of Sherman rats (60/sex/dose level) were treated with 1,4-dioxane in the drinking water at levels of 0 (controls), 0.01, 0.1, or 1% for 716 days. Based on body weight and water consumption data, the investigators estimated that the water provided doses of 1,4-dioxane of 0, 9.6, 94, and 1,015 mg/kg/day for males and 0, 19, 148, and 1,599 mg/kg/day for females. Blood samples were collected from controls and high-dose rats during the 4th, 6th, 12th, and 18th months of the study and at termination. Additional end points evaluated included clinical signs, body weight, organ weights, and gross and microscopic examination of major tissues and organs. Treatment with 1,4-dioxane significantly increased mortality in males dosed with 1,015 mg/kg/day and in females dosed with 1,599 mg/kg/day beginning at about 2–4 months of treatment. These rats showed degenerative changes in both the liver and kidneys. Rats in these groups also showed significantly reduced water consumption during the first year of the study and body weight gain was significantly reduced from the beginning of the study. Microscopic lesions were restricted to the liver and kidneys in males treated with ≥94 mg/kg/day and females dosed with ≥148 mg/kg/day. The liver lesions consisted of various degrees of hepatocellular degeneration and necrosis and evidence of hepatic regeneration as indicated by hepatocellular hyperplastic nodule formation. The NOAEL for liver effects was 9.6 mg/kg/day in males and 19 mg/kg/day in females. The LOAELs were 94 mg/kg/day in males and 148 mg/kg/day in females. The kidneys showed tubular epithelial degeneration and necrosis, and there was evidence of renal tubular regeneration as indicated by increased tubular epithelial regenerative activity (≥94 mg/kg/day in males and ≥148 mg/kg/day in females). There were no compound-related alterations in hematological parameters at any time point. The MRL of 0.1 mg/kg/day was calculated by dividing the male rat NOAEL of 9.6 mg/kg/day by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

The NOAEL and LOAEL for liver effects from Kociba et al. (1974) are supported by the results of JBRC (1998c). In that study, groups of Fischer 344/DuCrj rats (50/sex/dose level) received 1,4-dioxane in the drinking water for 104 weeks. 1,4-Dioxane was administered at levels of 0, 200, 1,000, and 5,000 ppm for 2 years (0, 16, 81, and 398 mg/kg/day for males; 0, 21, 103, and 514 mg/kg/day for females). End points evaluated included clinical signs, food and water consumption, body and organ weights, comprehensive hematology and clinical chemistry tests, urinalysis, and gross and microscopic examination of major organs and tissues. In males, relative liver weight was increased at ≥81 mg/kg/day and absolute liver weight was increased at 398 mg/kg/day. A significant increase incidence of spongiosis,

hyperplasia, and clear and mixed cell foci was observed in the liver from male rats with ≥81 mg 1,4-dioxane/kg/day, but not 16 mg/kg/day. These lesions were observed in females dosed with 514 mg/kg/day, but not with lower doses. In addition, in this study, female rats dosed with ≥103 mg 1,4-dioxane/kg/day showed nuclear enlargement of the olfactory epithelium of the nasal cavity; no such lesions occurred with the lower female rat dose of 21 mg/kg/day.

The NCI (1978) bioassay in Osborne-Mendel rats used somewhat higher dose levels than Kociba et al. (1974) and JBRC (1998c), but did not observe liver lesions in male rats dosed with 240 mg 1,4-dioxane/kg/day, a dose level that caused liver hyperplasia in male Fischer 344 rats dosed with 81 mg/kg/day or that caused hepatocyte degeneration in Sherman rats dosed with 94 mg/kg/day. Since the dosing method was the same in the three studies, the drinking water, the different results may reflect differences in strain sensitivity.

An alternate approach to derive a chronic-duration oral MRL is to use the PBPK model developed by Reitz et al. (1990), as was done above for the chronic inhalation data. Using the model, it can be estimated that the human equivalent dose to the NOAEL of 9.6 mg/kg/day for liver effects in males is 12.9 mg/kg/day. Applying an uncertainty factor of 30 (3 for using dosimetric adjustments and 10 for human variability) to the human equivalent NOAEL of 12.9 mg/kg/day yields a chronic-duration oral MRL of 0.4 mg/kg/day, which supports the MRL of 0.1 mg/kg/day derived above. A detailed explanation of the use of the model is presented in Appendix B.