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. 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.

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 (1.5–12 ppm in baby and children's products and 2–23 ppm in adult products) than in the survey done by the FDA in the 1990s.

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 kidney 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 either solely or in part to 1,4-dioxane.

Results from a recent 13-week study in rats and a 2-year study in rats indicate that the tissues in the nasal cavity are the most sensitive target for 1,4-dioxane following inhalation exposure. Adverse nasal effects were seen in rats exposed to ≥100 ppm in the 13-week study and in rats exposed to ≥50 ppm in the 2-year study. These exposure concentrations were the lowest tested. The liver and kidneys are also targets of 1,4-dioxane toxicity in animals 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 1,033 mg/kg/day, a dose level that also affected the mothers. Chronic inhalation exposure of male rats to 1,4-dioxane induced benign tumors in the liver (1,250 ppm but not 250 ppm), squamous cell carcinoma in the nasal cavity (1,250 ppm but not 250 ppm), and mesothelioma in the peritoneum (≥250 ppm but not 50 ppm). Chronic administration of 1,4-dioxane in the drinking water produced liver cancer in rats (range, 398–1,015 mg/kg/day), mice (range, 77–380 mg/kg/day), and guinea pigs (1,014 mg/kg/day), and cancer of the nasal cavity in rats (range, 429–833 mg/kg/day). However, a 2-year inhalation study in rats exposed to 111 ppm 1,4-dioxane (equivalent to oral doses of approximately 105 mg/kg/day), 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.

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 (1,428 mg/kg/day in rats) and dermal (143 mg/kg/day in guinea pigs; 57 mg/kg/day in rabbits) exposure. Hepatocyte vacuolation and swelling were reported in rats and mice dosed with 1,4-dioxane in the drinking water for 2 weeks (>2,500 mg/kg/day) or 13 weeks (≥126 mg/kg/day in rats; >550 mg/kg/day in mice). Evidence of hepatic degenerative changes was seen in Sherman rats that died after 2–4 months of receiving doses of 1,015 mg/kg/day 1,4-dioxane via the drinking water in a 2-year bioassay. Chronic inhalation exposure of male F344 rats to 1,250 ppm induced hepatic centrilobular necrosis and nuclear enlargement. Long-term oral studies in animals described hepatocellular degeneration and necrosis in Sherman rats at about 94 mg 1,4-dioxane/kg/day and increased cell foci in F344 rats at ≥55 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. Conversely, 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, generally in the high-dose groups. In the better reported studies, tumor development occurred at doses that produced extensive liver toxicity, including hepatocellular hyperplasia and degeneration and evidence of hepatic regeneration, which has led some to suggest that cell damage and degeneration may be a necessary occurrence for the formation of liver tumors in rats. Oral exposure to 1,4-dioxane also induced tumors in the nasal cavity in rats and liver tumors in mice and guinea pigs. The relevance of the nasal tumors to humans following oral exposure to 1,4-dioxane has been questioned and some scientists suggested that the tumors resulted from inspiration of water containing 1,4-dioxane into the nasal cavity. A study reported that the addition of a fluorescent dye mixture to water containing 0.5% 1,4-dioxane and offered to rats as drinking water resulted in the fluorescent dye readily observed in numerous areas in the nasal cavity where bioassays have identified tumors. Little or no fluorescence associated with the dye mixture was found in a single rat that received the dye mixture by gavage. One study concluded that these results indicate that the rat nasal tissues are exposed by direct contact with drinking water under conditions of the bioassay. However, there is also evidence in support of the nasal alterations being caused, at least in part, by systemic delivery of either 1,4-dioxane or a metabolite (see Section 3.5.2). 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, whether by direct contact alone or a combination of direct contact and internal exposure.

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 oral exposure to 1,4-dioxane, last revised in 2010, based on the increased incidence of hepatocellular adenoma and carcinomas in female Crj:BDF_1 mice in a 2-year drinking-water bioassay.

Liver toxicity has been proposed to be necessary for liver tumor formation 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. 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 likely to be carcinogenic to humans based on inadequate evidence of carcinogenicity in humans and sufficient evidence in animals.

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 (≥5,000 ppm) showed severe kidney damage consisting of 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 to 1,000 ppm 1,4-dioxane for up to 12 weeks. Similar observations were made in intermediate-duration studies in rats and mice exposed orally (1,400–2,900 mg 1,4-dioxane/kg/day) and in guinea pigs (143 mg/kg) and rabbits (57 mg/kg) 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 with 1,015 mg 1,4-dioxane/kg/day in a 2-year drinking water bioassay. Nuclear enlargement of the proximal tubule was reported in rats exposed to 657 mg 1,4-dioxane/kg/day in a 13-week study. Increased incidence of degeneration and necrosis of the tubular epithelium was seen in rats that received 94 mg/kg/day and survived until termination of the study, and similar findings were reported in Osborne-Mendel rats that received 240 mg/kg/day. Nuclear enlargement in the proximal convoluted tubule was reported in male F344 rats exposed to ≥250 ppm 1,4-dioxane vapors for 2 years. 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 oliguria and acute renal failure. 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 forced 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 of exposure. The NOAEL of 20 ppm was divided by an uncertainty factor of 10 (for human variability) to yield the MRL of 2 ppm.

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 only complaint throughout the exposure. Perception of the odor of 1,4-dioxane diminished with time. 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).

- An MRL of 0.2 ppm has been derived for intermediate-duration inhalation exposure (15–364 days) to 1,4-dioxane.

The intermediate-duration database for 1,4-dioxane consists of only two studies: an early study that exposed several animal species to high concentrations of 1,4-dioxane and monitored limited end points (Fairley et al. 1934); and a recent study that evaluated a comprehensive number of end points in rats exposed to several concentrations of 1,4-dioxane (Kasai et al. 2008). Fairley et al. (1934) exposed rats, mice, guinea pigs, and rabbits to airborne 1,4-dioxane 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. In the recent study, groups of F344/DuCrj rats (10/sex/group) were exposed to target concentrations of 0 (clean air), 100, 200, 400, 800, 1,600, 3,200, or 6,400 ppm 1,4-dioxane vapors 6 hours/day, 5 days/week for 13 weeks (Kasai et al. 2008). End points evaluated included mortality, clinical signs (daily), body weight and food consumption (once per week), hematology, clinical chemistry and urinalysis at termination, and gross and microscopic pathology of all major organs and tissues. All rats in the 6,400 ppm group died during the first week of the study. Examination of these rats showed that death was primarily caused by renal failure, as judged by marked necrosis observed in the renal tubules. Lung congestion was also observed in males and females from this exposure group. At the remaining exposure concentrations, no abnormal clinical signs were observed during the study. Terminal body weight was reduced in all treated groups except the 100 ppm group; the final weight was reduced more than 10% relative to controls only in females exposed to 3,200 ppm. Data on food consumption were not provided. Significant changes in organ weight (>10% difference with controls) were limited to the liver, kidneys, and lungs and consisted of increases in relative organ weight, generally in the high dose groups of up to 15% relative to controls; data on absolute organ weights were not provided. Significant changes (although within normal values) in hematology and clinical chemistry parameters were limited to the 3,200 ppm groups and consisted of increases in mean corpuscular volume and serum alanine aminotransferase (ALT) in males, decrease in glucose and triglycerides in males, and increases in red blood cell count, hemoglobin, hematocrit, and aspartate aminotransferase (AST) and ALT serum activities in females. Histologically, exposure to 1,4-dioxane affected principally the respiratory tract, in particular the nasal cavity of males and females. Significant nuclear enlargement of the respiratory epithelium was seen in all exposed groups. The incidences in males were 0/10 in the control group and 7/10, 9/10, 7/10, 10/10, 10/10, and 10/10 in the exposed groups up to 3,200 ppm, respectively. The corresponding incidences in females were 0/10, 5/10, 9/10, 10/10, 10/10, 10/10, and 10/10. The severity of the lesion was concentration-related. Significant nuclear enlargement of the olfactory epithelium started at 200 ppm (5/10 in males and 6/10 in females). Similar lesions in the trachea and bronchus

appeared only in the high-exposure groups. The nuclear enlargement was characterized by the epithelial cells having a round to oval or elongated nucleus at least 4 times larger in diameter than normal. Significantly increased incidence of vacuolic change in the olfactory epithelium started in males at 400 ppm (0/10, 1/10, 3/10, 6/10, 10/10, 9/10) and in females at 800 ppm (0/10, 1/10, 2/10, 3/10, 7/10, 9/10, 10/10), while atrophy of the olfactory epithelium started in females at 800 ppm (0/10, 0/10, 2/10, 3/30, 5/10, 5/10, 4/10) and none was seen in males. Significant single cell necrosis and centrilobular swelling occurred in the liver of males exposed to 3,200 ppm 1,4-dioxane; females in this exposure group showed only centrilobular swelling. Significant kidney changes were seen only in females from the 3,200 ppm exposure group and consisted of hydropic changes in the proximal tubule. No treatment-related lesions were reported in any other tissue or organ examined.

Although nuclear enlargement of the respiratory and olfactory epithelium occurred at lower exposure levels than other nasal lesions, it was not selected as the critical effect for MRL derivation on the grounds that the toxicological significance of the lesion is uncertain. There is some evidence, although not conclusive at this time, suggesting that this alteration may represent a preneoplastic lesion. Indeed, preneoplastic and neoplastic lesions were observed in rats exposed chronically in the Kasai et al. (2009) study. Furthermore, as discussed by Kasai et al. (2008), nuclear enlargement occurred as an early histopathological change in the respiratory tract of rats simultaneously exposed to sulfur dioxide and treated intraperitoneally with several N-nitrosamines known to induce nasal tumors in rats (Fowlie et al. 1990). In addition, studies have shown a good correlation between *in vivo* carcinogenicity and the extent of nuclear enlargement in HeLa cells *in vitro* (Grant and Grasso 1978). Since MRLs are not based on a consideration of cancer effects, nuclear enlargement is not considered an appropriate basis for MRL derivation.

Incidence data for vacuolic change in the olfactory epithelium in male and female rats and for atrophy of the olfactory epithelium in female rats exposed to 1,4-dioxane vapors (Kasai et al. 2008) were analyzed using the benchmark dose/concentration (BMD/BMC) approach for MRL derivation (further details of the modeling are presented in Appendix A). A multistage (1-degree) model provided the best fit to the vacuolic change in both the male and female data, whereas a log-logistic model provided the best fit for the atrophy of the olfactory epithelium in female rats. From these models, the lowest predicted exposure concentrations associated with a 10% extra risk (BMC_{10}) was 40.39 ppm and corresponded to vacuolic changes in the respiratory epithelium of male rats; the corresponding lower 95% confidence limits on this concentration ($BMCL_{10}$) was 27.99 ppm.

The $BMCL_{10}$ of 27.99 ppm was converted to a human equivalent exposure concentration (HEC) ($BMCL_{10[HEC]}$) in consideration of EPA (1994) cross-species dosimetric methodology for inhaled gases. Although 1,4-dioxane produces portal-of-entry effects typical of a category 1 gas, it deviates from the strict definition of a category 1 gas in that: (1) it is not potently reactive, (2) it enters the systemic circulation via portal-of entry tissues, and (3) it induces systemic effects following inhalation exposure (i.e., liver and kidney effects). Both (1) and (3) are consistent with category 3 gases; however, atypical of a category 3 gas, 1,4-dioxane's critical effect from repeated inhalation exposure is a portal-of-entry effect (nasal lesions). 1,4-Dioxane may be more appropriately classified as a category 2 gas (moderately water-soluble and moderately to slowly reactive in respiratory tissue; EPA 1994); however, the EPA (1994) default dosimetric equations for respiratory effects from category 2 gases have not been widely used because they lead to human equivalent concentrations that are orders of magnitude lower than concentrations administered to rodents. Dosimetric equations for a category 1 gas (based on a ratio of the ratios of ventilation rate to extrathoracic surface area in rats to humans) indicate that effects observed in rodents at a specific air concentration will occur in humans at lower air concentrations (i.e., a $LOAEL_{human}$ will be about 0.25 x a $LOAEL_{rat}$, or the nasal dose experienced by humans at a specific air concentration will be higher than nasal doses experienced by rodents). In contrast, dosimetric equations for category 3 gases indicate that steady-state blood concentrations (and nasal doses) are determined principally by blood:air coefficients, and that, for chemicals like 1,4-dioxane with higher blood:air coefficients in rats (1,861; Sweeney et al. 2008) than in humans (1,666; Sweeney et al. 2008), effects observed in rats at a specific air concentration will occur in humans at equivalent or higher air concentrations (EPA 1994). This means that at a specific air concentration, nasal doses in humans would be equivalent to or less than nasal doses in rats. More sophisticated dosimetric cross-species extrapolation approaches have been developed for several other nasal toxicants linking computerized fluid dynamic (CFD) nasal airflow patterns and species-specific anatomical features with physiologically based pharmacokinetic (PBPK) models incorporating species-specific information about diffusion, metabolism, and kinetic distribution of the agents among tissues. Predictions from these models indicate that, at a specific air concentration, nasal doses would be nearly equivalent between humans and rats (e.g., vinyl acetate [Andersen et al. 2002]) or would be lower in humans than in rats (methyl acrylate [Andersen et al. 2002]; ethyl acrylate [Sweeney et al. 2004]; and formaldehyde [Conolly et al. 2004; Kimbell et al. 2001]). Because of these predictions, it is expected that the category 3 gas dosimetric equation is more appropriate for 1,4-dioxane than the category 1 gas dosimetric equation. Thus, the EPA (1994) category 3 gas dosimetric equation was used in deriving the MRL, in addition to a duration adjustment (6/24 hours x 5/7 days), which was considered appropriate in the absence of information regarding whether Haber's Law is applicable under the experimental conditions of the study.

The MRL is derived as follows:

$$BMCL_{10[HEC]} = BMCL_{10[ADJ]} \times (H_{b/g}A / H_{b/g}H)$$

where:
$BMCL_{10[ADJ]}$ = 27.99 ppm x 6/24 hours x 5/7 days = 4.998 ppm and
$H_{b/g}A$ = animal blood:air partition coefficient = 1,861 (Sweeney et al. 2008)
$H_{b/g}H$ = human blood:air partition coefficient = 1,666 (Sweeney et al. 2008)

$$(H_{b/g}A / H_{b/g}H) = 1{,}861/1{,}666 = 1.117$$

Because the ratio of the partition coefficients is higher than 1, a default value of 1 is used in accordance with EPA's RfC methodology (EPA 1994).

$$BMCL_{[HEC]} = 4.998 \text{ ppm} \times 1 = 4.998 \text{ ppm}$$

Applying an uncertainty factor of 30 (3 for extrapolation from animals to humans with dosimetric adjustment and 10 for human variability) to the $BMCL_{[HEC]}$, rounded to the nearest single digit, yields an intermediate-duration inhalation MRL of 0.2 ppm for 1,4-dioxane.

- An MRL of 0.03 ppm has been derived for chronic-duration inhalation exposure (365 days or more) to 1,4-dioxane.

The chronic inhalation database for 1,4-dioxane in humans and animals is limited. 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. These studies were not considered for MRL derivation because they do not provide enough information to verify the conclusions of the authors.

Two chronic-duration inhalation studies in animals are available for 1,4-dioxane (Kasai et al. 2009; Torkelson et al. 1974). In a study conducted by Torkelson et al. (1974), 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 ALT and AP 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. It should be noted, however, that the tissues from the nasal cavity were not listed among the tissues that were subjected to microscopic examination by Torkelson et al. (1974), although in the discussion of the results, the investigators state that no nasal tumors were observed in any rats. Although there were no clinical signs, early mortality, nasal tumors, or any other indication that the health of the rats was compromised in the 2-year study, there is uncertainty regarding the possibility that the nuclear enlargement observed in the 13-week study might progress to cancer.

Kasai et al. (2009) whole-body exposed groups of male F344/DuCrj rats (50/group) to target concentrations of 0, 50, 250, or 1,250 ppm 1,4-dioxane vapors 6 hours/day, 5 days/week for 104 weeks; controls were exposed to clean air. End points evaluated included clinical signs and mortality (daily) and body weight and food consumption (once/week for the first 14 weeks, every 4 weeks thereafter). All rats were subjected to complete necropsy. Blood was collected at termination for clinical chemistry and hematology tests; urinary pH was measured in the last week of the study. All major organs were removed, weighed, and examined for macroscopic lesions. All major tissues and organs, including the entire respiratory tract, were examined microscopically. Survival rates in rats exposed to 250 ppm tended to decrease relative to controls, but the difference with controls was not statistically significant. Exposure to 1,250 ppm 1,4-dioxane significantly reduced ($p<0.05$) survival rate beginning on week 91. Terminal survival rate was 37/50, 37/50, 29/50, and 25/50 in the control, low-, mid-, and high-exposure groups, respectively. The decrease in survival rates was attributed to increased number of deaths due primarily to peritoneal mesotheliomas, although nasal tumors contributed to the causes of death. Terminal body weight was reduced 6.3% in the high-exposure group. Food consumption was not affected by exposure to 1,4-dioxane. Statistically significant increases in relative liver (27%) and lung (2%) weights were reported in the high-exposure group, but there was no clear dose-response relationship. Significant changes in hematology and clinical chemistry tests included reduced hemoglobin (13%), mean corpuscular volume (MCV, 6%), mean corpuscular hemoglobin (MCH, 8%), increased serum AST (46%), ALT (95%), AP (15%), and gamma-glutamyl transpeptidase (γ-GTP, 6–7-fold); urinary pH was

reduced 7%. All of these changes were restricted to the high-exposure group. Treatment-related pre- and nonneoplastic lesions occurred in the nasal cavity, liver, and kidney. All exposed groups had significant increases in nuclear enlargement of the respiratory epithelium (0/50, 50/50, 48/50, 38/50), nuclear enlargement of the olfactory epithelium (0/50, 48/50, 48/50, 45/50), atrophy of the olfactory epithelium (0/50, 40/50, 47/50, 48/50), and respiratory metaplasia of the olfactory epithelium (11/50, 34/50, 49/50, 48/50). Incidences of hydropic change and sclerosis of the lamina propia were significantly increased only in the mid- and high-exposure groups. Significant increases in liver lesions (centrilobular nuclear enlargement, acidophilic cell foci, basophilic cell foci, spongiosis hepatis, and centrilobular necrosis) occurred in the high-exposure group. Significant increases in nuclear enlargement of the proximal kidney tubule occurred in the mid- and high-exposure groups; significantly increased incidence of hydropic changes in the proximal tubule occurred in the high-exposure group. No significant changes occurred in other organs or tissues. The lowest exposure concentration tested, 50 ppm 1,4-dioxane, is a LOAEL for nasal lesions (atrophy of the olfactory epithelium); a NOAEL was not defined in this study.

Of the two available studies, the study by Kasai et al. (2009) was selected for MRL derivation because it conducted a complete examination of the respiratory tract, including nasal passages, plus Torkelson et al. (1974) established a free-standing NOAEL. The results of Kasai et al. (2009) clearly show that the nasal cavity was the most sensitive tissue following 2 years of exposure to 1,4-dioxane vapors. As discussed in the derivation of the intermediate-duration inhalation MRL for 1,4-dioxane, nuclear enlargement was not considered as the basis of an MRL because of evidence suggesting that the alteration may represent a pre-neoplastic lesion. Incidences of atrophy (0/50, 40/50, 47/50, and 48/50) and respiratory metaplasia (11/50, 34/50, 49/50, and 48/50) of the olfactory epithelium were also significantly elevated at all exposure levels tested. Of these two lesions, the atrophy of the olfactory epithelium was selected as the critical effect for MRL derivation because it showed a higher incidence rate at the LOAEL than respiratory metaplasia. Because the incidence of this lesion at the lowest exposure level (50 ppm) was close to the maximal response level (80% of 50-ppm animals showed this lesion), BMD analysis of the data was not conducted. This decision is in accordance with guidelines stating that studies in which responses are at or near the maximal response level are not considered adequate for BMD analysis (EPA 2000a).

The LOAEL of 50 ppm was converted to a HEC using the EPA cross-species dosimetric methodology (EPA 1994) for a category 3 gas as explained in the derivation of the intermediate-duration inhalation MRL. A duration adjustment (6/24 hours x 5/7 days) seemed appropriate in the absence of information

regarding whether Haber's Law is applicable under the experimental conditions of the study. The MRL is derived as follows:

$$LOAEL_{[HEC]} = LOAEL_{[ADJ]} \times (H_{b/g}A / H_{b/g}H)$$

where:
$LOAEL_{[ADJ]}$ = 50 ppm x 6/24 hours x 5/7 days = 8.9286 ppm and
$H_{b/g}A$ = animal blood:air partition coefficient = 1,861 (Sweeney et al. 2008)
$H_{b/g}H$ = human blood:air partition coefficient = 1,666 (Sweeney et al. 2008)

$$(H_{b/g}A / H_{b/g}H) = 1{,}861/1{,}666 = 1.117$$

Because the ratio of the partition coefficients is higher than 1, a default value of 1 is used in accordance with EPA's RfC methodology (EPA 1994).

$$LOAEL_{[HEC]} = 8.9286 \text{ ppm} \times 1 = 8.9286 \text{ ppm}$$

Applying an uncertainty factor of 300 (10 for use of a LOAEL, 3 for extrapolation from animals to humans with dosimetric adjustment, and 10 for human variability) to the $LOAEL_{[HEC]}$ yields a chronic-duration inhalation MRL of 0.03 ppm for 1,4-dioxane.

Oral MRLs

- An MRL of 5 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to 1,4-dioxane.

Only two acute-duration oral studies potentially useful for MRL derivation are available for 1,4-dioxane. JRBC (1998) conducted a 2-week drinking water study in F344 rats and $B6C3F_1$ mice and reported that the most sensitive effect was an increased incidence of nuclear enlargement of the olfactory epithelium in male and female rats receiving doses of approximately 1,010 and 1,040 mg 1,4-dioxane/kg/day, respectively; the corresponding NOAELs were 370 and 400 mg/kg/day. Liver and kidney alterations were observed at higher doses. Giavini et al. (1985) administered 1,4-dioxane by gavage in water to pregnant Sprague-Dawley rats on gestation days (Gd) 6 through 15 and reported slight but significant reductions in fetal weight and in the percent of ossified sternebrae in the group treated with the highest dose of 1,4-dioxane, 1,033 mg/kg/day; the NOAEL was 516 mg/kg/day. Most of the rest of the acute database consists of high-dose early studies aimed mainly at determining LD_{50} values and inadequate for risk assessment (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 resulted in the death of one dog after 10 days of treatment. Doses of 375 mg/kg/day resulted in the death of 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.

Of the two potentially useful studies for MRL derivation mentioned above, the developmental study conducted by Giavini et al. (1985) is preferred as basis for the acute-duration oral MRL for 1,4-dioxane mainly due to overall deficiencies in the JRBC (1998) study, namely, lack of statistical analysis of the results due to the fact that only 2 or 3 animals (out of 10/group) were examined, and the fact that end points such as hematology, clinical chemistry, clinical signs, and gross examinations were not conducted or reported. These limitations severely compromise the interpretation of the results.

In the study selected as basis for the acute oral MRL, groups of 17–20 pregnant Sprague-Dawley rats were treated with 0, 0.25, 0.5, or 1 mL 1,4-dioxane/kg/day (0, 258, 516, or 1,033 mg 1,4-dioxane/kg/day based on a specific gravity of 1.034) by gavage in water on Gd 6–15 (Giavini et al. 1985). Food consumption was determined daily and body weight was monitored every three days. Sacrifices were conducted on Gd 21 and the number of corpora lutea, implantations, resorptions and live fetuses was recorded. The fetuses were weighed and inspected for external malformations and half were examined for visceral abnormalities and the other half for skeletal malformations. Rats treated with 1,033 mg 1,4-dioxane/kg/day gained 18% less weight than controls during treatment days, although the difference was not statistically significant. Food consumption was slightly (5%) but significantly ($p<0.05$) reduced in these rats during treatment. The average fetal weight in the high-dose group was slightly but significantly ($p<0.01$) lower than in controls. Also, a slight but significant ($p<0.05$) reduction in sternum ossification was seen in high-dose fetuses. There were no significant effects on the number of implantations and live fetuses, post-implantation loss, or incidence of malformations. Based on the reduced maternal and fetal body weight and reduced sternum ossification, a maternal and developmental LOAEL of 1,033 mg 1,4-dioxane/kg/day can be defined; the maternal and developmental NOAEL is 516 mg/kg/day. Attempts made to apply dose-response models to the data were unsuccessful, as no adequate fits of EPA BMDS models to the data were obtained; therefore, the NOAEL/LOAEL approach was used for MRL derivation. An acute-duration oral MRL of 5 mg/kg/day was derived for 1,4-dioxane by dividing the NOAEL of 516 mg/kg/day by an uncertainty factor of 100 (10 for animal to human extrapolation and 10 for human variability).

- An MRL of 0.5 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 52 mg 1,4-dioxane/kg/day for liver effects in rats (Kano et al. 2008). 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, 52, 126, 274, 657, or 1,554 mg/kg/day in males; 0, 83, 185, 427, 756, or 1,614 mg/kg/day in females, estimated by the investigators). 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 1,614 mg/kg/day group died. Body weight gain was reduced at 756 mg/kg/day (12%) and 1,614 mg/kg/day (21%) in females and at 1,554 mg/kg/day (21%) in males. Food consumption was reduced 13% in females at 1,614 mg/kg/day. Water consumption was reduced in a dose-related manner in all male groups and in females at ≥126 mg/kg/day. Hematology tests showed significant increases in erythrocyte counts, hemoglobin, hematocrit, and neutrophils, and a decrease in lymphocytes in males at 1,554 mg/kg/day, and decreases in mean corpuscular volume and platelets in females at 1,614 mg/kg/day. Total protein and albumin were decreased in males at ≥274 mg/kg/day and in females at ≥427 mg/kg/day. Serum 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 ≥274 mg/kg/day and in females at ≥756 mg/kg/day. Absolute and relative kidney weights were increased in females at ≥231 mg/kg/day. Nuclear enlargement of the respiratory epithelium occurred in males at ≥126 mg/kg/day and in females at ≥185 mg/kg/day; nuclear enlargement of the olfactory and tracheal epithelium occurred in males at ≥274 mg/kg/day and in females at ≥427 mg/kg/day. Swelling of the central area of the liver was observed in males at ≥126 mg/kg/day and in females at ≥756 mg/kg/day, and vacuolar changes in the liver occurred in males at ≥657 mg/kg/day and in females at 1,614 mg/kg/day. The incidences of swelling of the central area of the liver in males were 0/10, 0/10, 9/10, 10/10, 10/10, and 10/10 in the control, 52, 126, 274, 657, and 1,554 mg/kg/day dose groups, respectively. Nuclear enlargement of the proximal tubule of the kidneys was seen in males at ≥657 mg/kg/day and in females at ≥756 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 (1,554 and 1,614 mg/kg/day, respectively). The study LOAEL was 126 mg/kg/day for liver 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 52 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 0.5 mg/kg/day. The steepness of the dose-response relationship for liver lesions rendered the data set inadequate for BMD analysis.

A promotion study by Lundberg et al. (1987) supports the liver findings of Kano et al. (2008). 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 sacrificed, 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. Because of the limited scope of the study by Lundberg et al. (1987) (only the liver was examined) and because the NOAEL of 100 mg/kg/day is practically the same as the LOAEL of 126 mg/kg/day identified by Kano et al. (2008), the latter study was preferred for MRL derivation. Also supporting the findings from Kano et al. (2008) 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).

Although available rat and mouse PBPK models (Leung and Paustenbach 1990; Reitz et al. 1990; Sweeney et al. 2008) provide adequate fits of high-dose observations, they do not perform well against low-dose data; thus, they were not used for MRL derivation. The human model could not replicate the limited human experimental inhalation data available (Sweeney et al. 2008). Further, it assumes equivalency with mice in eliminating β-hydroxyethoxyacetic acid (HEAA), and has no value derived for oral absorption. Based on these significant limitations, the Sweeney et al. (2008) model for 1,4-dioxane in rats, mice, and humans, which is a more refined version of the earlier models, is not adequate for MRL derivation. This applies also to the use of PBPK models for derivation of the chronic-duration oral MRL for 1,4-dioxane described below.

- 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 in other groups 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 lack of quantitative information regarding incidences of non-neoplastic lesions precludes the use of BMD methodology for MRL derivation.

The NOAEL and LOAEL for liver effects from Kociba et al. (1974) are supported by the results of Kano et al. (2009). In that study, groups of F344/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, 11, 55, and 274 mg/kg/day for males; 0, 18, 83, and 429 mg/kg/day for females). End points evaluated included clinical signs, food and water consumption, body and organ weights, and gross and microscopic examination of major organs and tissues. Terminal body weight was reduced 9% in high-dose males (274 mg/kg/day) and 20% in high-dose females (429 mg/kg/day). In males, relative liver weight was significantly increased at 55 mg/kg/day (14%) and 274 mg/kg/day (72%). A significant increase incidence of mixed cell foci was observed in the liver from male rats dosed with ≥55 mg 1,4-dioxane/kg/day. Increased incidence of acidophilic and mixed cell foci were reported in the liver from high-dose females (429 mg/kg/day). In addition, both high-dose male (274 mg/kg/day) and female (429 mg/kg/day) rats had significantly increased incidence of nuclear enlargement and squamous cell metaplasia of the respiratory epithelium; females dosed with ≥83 mg 1,4-dioxane/kg/day also showed a significantly increased incidence of nuclear enlargement of the nasal olfactory epithelium.

The NCI (1978) bioassay in Osborne-Mendel rats used somewhat higher dose levels than Kociba et al. (1974) and Kano et al. (2009), but did not observe liver lesions in male rats dosed with 240 mg 1,4-dioxane/kg/day. However, a dose level of 55 mg/kg/day induced cell foci in the liver from male F344 rats, and 94 mg/kg/day caused hepatocyte degeneration in Sherman rats. Since the dosing method was the same in all three studies (via the drinking water) the different results may reflect differences in strain sensitivity.