APPENDIX A
ATSDR MINIMAL RISK LEVEL

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99-4991, requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the US. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (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 for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1-14 days), intermediate (15-364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

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MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL, may be as much as a hundredfold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology, expert panel peer reviews, and agencywide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road, Mailstop E-29, Atlanta, Georgia 30333.

APPENDIX A

Chemical name(s): 2-Butoxyethanol
CAS number(s): 111-76-2
Date: June 1998
Profile status: Draft 3
Route: [X] Inhalation [ ] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Key to figure: 21
Species: Rat

MRL: 6 [ ] mg/kg/day [X] ppm [ ] mg/m^3

Reference: Tyl et al. 1984

Experimental design: Timed-pregnant Fischer 344 rats (n=36) were exposed to 2-butoxyethanol vapors by inhalation on Gd 6–15 at concentrations 0, 25, 50, 100, or 200 ppm for 6 hours per day (Tyl et al. 1984). The animals were observed for clinical signs throughout the study, and food and water consumption (withheld during exposures) were measured. Maternal body weights were taken on Gd 0, 6, 9, 12, 15, and 21. The animals were sacrificed on Gd 21 after obtaining blood samples. Hematology and organ weights (uterus, liver, thymus, spleen, and kidney) were evaluated. Hematologic determinations on dams showed significant reductions in erythrocyte (RBC) count, mean corpuscular hemoglobin concentration (MCHC), and an increase in hemoglobin per cell (MCH) and size of the red blood cell (MCV) at 100 and 200 ppm exposure, but not at 50 ppm. There were significant increases in hemoglobin and hematocrit counts at 200 ppm. Clinical signs that occurred with an incidence different from the controls were observed in 75% of dams in the 200 ppm group during the exposure period, and included the clear evidence of hematuria or hemoglobinuria, periocular wetness, perinasal encrustation, pale and cold extremities, and necrosis of the tail tip. Periocular wetness, clear evidence of hematuria, and perinasal discharge were also noted in 25–50% of the dams in the 100 ppm dose group. In the 25 and 50 ppm dose groups, 25% of the dams showed signs of periocular wetness. Maternal body weight gain was decreased 29% in the 100 ppm group at Gd 6–15, compared to the control group. Exposure to 200 ppm resulted in negative weight gain and decreased (at Gd 6–21) maternal body weight (11–12% at 200 ppm), decrease in food consumption at Gd 6–15 during treatment (13% decrease at 100 ppm), and evidence of anemia. Decreased water consumption (14%) was noted at 200 ppm. There was a reduction in maternal gravid uterine weight at 200 ppm. Relative kidney weight was elevated as compared to controls at 200 ppm in the presence of decreased body weight. No treatment-related effects were observed on the weights of the liver.

Effects noted in study and corresponding doses: A clear dose response relationship was observed for hematological and renal toxicity:

50 ppm = low dose (NOAEL)

100 ppm = mid dose (Serious LOAEL: females showed decreased erythrocyte (RBC) count, mean corpuscular hemoglobin concentration (MCHC), an increase in hemoglobin per cell (MCH) and size of the red blood cell (MCV); clear evidence of hematuria noted in 25–50% of the dams)

200 ppm = high dose (decreased erythrocyte (RBC) count, mean corpuscular hemoglobin concentration (MCHC), an increase in hemoglobin per cell (MCH) and size of the red blood cell (MCV); significant increases in hemoglobin and hematocrit counts; clear evidence of hematuria or hemoglobinuria in 75% of the dams)

Dose endpoint used for MRL derivation: 50 ppm - hematological effects

[X] NOAEL [ ] LOAEL

Uncertainty factors used in MRL derivation: 9

[ ] 1 [ ]3 [ ] 10 (for use of a LOAEL)
[ ] 1 [X] 3 [ ] 10 (for extrapolation from animals to humans)
[ ] 1 [X] 3 [] 10 (for human variability)

An uncertainty factor of 3 for animal to human extrapolation because *in vitro* data show that human red blood cells are much less sensitive to the hemolytic effects of the 2-BUTOXYETHANOL metabolite, 2-butoxyacetic acid.
Concentrations of 2-butoxyacetic acid that caused hemolysis of rat red blood cells did not cause hemolysis of human red blood cells (Bartnik et al. 1987). For example, following a 60 minute exposure of rat red blood cells to 7.5 mM 2-butoxyacetic acid 100% hemolysis was observed. Following a 60 minute exposure of human red blood cells to 15 mM 2-butoxyacetic acid, no hemolysis was observed (Bartnik et al. 1987). An *in vitro* study measuring more sensitive endpoints of red blood cell effects, mean cell volume and hematocrit, has also shown rat red blood cells to be more sensitive to 2-butoxyacetic acid compared to human red blood cells (Ghanayem and Sullivan 1993). For example, following a 1 hour exposure to 2 mM 2-butoxyacetic acid, mean corpuscular volume was about 150% of controls in rat red blood cells, and 105% of controls in human red blood cells, hematocrit was about 155% of controls in rat red blood cells, and 105% of controls in human red blood cells. Red blood cells of rabbits, hamsters, mice and baboons were also sensitive to the red blood cell effects of 2-butoxyacetic acid, while red blood cells of cats, pigs, dogs, and guinea pigs were not sensitive the effects of 2-butoxyacetic acid (Ghanayem and Sullivan 1993). This *in vitro* data is also supported by the Carpenter et al. (1956) study in which erythrocyte osmotic fragility, an effect observed in animals, was not observed in humans exposed by inhalation to ≤ 195 ppm 2-butoxyethanol for 4-8 hours. In addition, an *in vitro* study showed that human erythrocytes were resistant to the hemolytic effects of 2-butoxyacetic acid, the hemolytic metabolite of 2-butoxyethanol (Udden 1994).

An uncertainty factor of 3 for human variability was used because the results of *in vitro* data suggest that 2-butoxyethanol does not cause significant hemolysis of normal and potentially susceptible erythrocytes (Udden 1996). Udden (1994) reported that red blood cells in humans (including the elderly and patients with hereditary spherocytosis and sickle cell disease) were not susceptible to 2-butoxyacetic acid-induced hemolysis .

Was a conversion factor used from ppm in food or water to a mg/body weight dose?
If so, explain: Not applicable.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: The human equivalent dose (HEC) was calculated using Formula 4-48 from Interim Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry, EPA (1994). Though 2-butoxyethanol is a Category 2 gas (i.e., water soluble and accumulates in blood), the equation in the EPA (1994) document for extrarespiratory effects of Category 2 gases is presently under review and the recommended equation is that for Category 3 gases:

$$NOAEL_{[HEC]} = NOAEL_{[ADJ]} \times (H_{b/g})_A / (H_{b/g})_H$$

where:

$NOAEL_{[HEC]}$ = the NOAEL human equivalent concentration

$NOAEL_{[ADJ]}$ = the NOAEL adjusted for duration (see below)

$(H_{b/g})_A$ = blood/gas partition coefficient for animals

$(H_{b/g})_H$ = blood/gas partition coefficient for humans

$(H_{b/g})_A$ = not available

$(H_{b/g})_H$ = 7965 (Johanson and Dynesius 1988).

Because a blood/gas partition coefficient was not available for rats, a default of 1 was used for the ratio of partition coefficients.

Thus,

$NOAEL_{[HEC]}$ = 50 ppm x (1)

$NOAEL_{[HEC]}$ = 50 ppm

Was a conversion used from intermittent to continuous exposure? If so, explain: No adjustment for intermittent exposure was used because 2-butoxyethanol and its major metabolite, 2-butoxyacetic acid, are eliminated from the body within 24 hours. In addition, because of the high blood/gas partition coefficient (7965) relative to a low fat/blood partition coefficient (0.77 Jepson et al. 1992) blood concentrations would be expected to reach periodicity (i.e., the periodic steady state is such that the concentration versus time profile is the same for every week) during acute duration exposure (EPA 1994).

Other additional studies or pertinent information that lend support to this MRL: Although in the Tyl et al. (1984) study, periocular wetness was observed at high incidences in the rats at ≥25 ppm, indicating that 25 ppm is a minimal LOAEL for eye irritation (from direct contact of the eyes with the vapor during inhalation exposure), hemolysis is the most consistently observed critical effect of concern for exposure to 2-butoxyethanol. Furthermore, the use of 25 ppm as a minimal LOAEL for eye irritation would result in an acute MRL lower than the intermediate inhalation MRL (see Work Sheet below).

That hemotoxicity is a critical end point of concern is supported by other acute inhalation studies. In Dodd et al. (1983), Fischer 344 rats (8 male, 8 female) were exposed for 9 days, 6 hours per day, 5 days per week to 2-butoxyethanol target concentrations of 0, 25, 100, or 250 ppm (mean average concentrations determined by gas chromatography to be 0, 20, 86, or 245 ppm, respectively). Rats were observed for signs of toxicity during exposure and 14 days postexposure. Necropsies were performed on animals that died and on survivors killed at the end of the observation period. Biological assessments included daily animal observations, body weight and organ (kidney, liver, lungs, and testes) weight determinations and ophthalmologic, hematologic, and gross pathologic examinations. The mean chamber concentrations of 25, 100, and 250 ppm 2-butoxyethanol (BE) were determined by gas chromatography to be 20, 86, and 245 ppm, respectively. A few male rats exposed to 245 ppm were observed to have audible respiration and nasal discharge. There was no effect on the hematologic parameters in rats exposed to 20 ppm BE (NOAEL). At 86 ppm exposure (LOAEL), in both sexes there was a significant, but less profound, effect on the erythroid parameters. At 245 ppm exposure, both male and female rats showed significantly depressed red blood cells (RBC) of 21% below control values, hemoglobin (HGB) and mean corpuscular concentration (MCHC) and significant increases in mean corpuscular volume (MCV), nucleated RBC, and reticulocytes. Red-stained urine was also

observed in rats of both sexes exposed to 245 ppm, but this staining was never observed again. Female rats exposed to 86 ppm showed significantly higher liver weights. Male and female rats of the 245 ppm group also showed increased liver weights. Decreased mean body weight gains (% not provided) were also found. A 14-day postexposure recovery showed substantial reversal of the affected blood parameters. In another study, nonpregnant rats were exposed to 250 ppm or greater 2-butoxyethanol by inhalation for 6.5 hours (Nelson et al. 1984). The exposed rats exhibited hematuria 4-6 hours after onset of exposure at all concentrations (LOAEL = 250 ppm). Surviving rats in each dose group had necrotic tail tips 1 week after exposure. New Zealand White rabbits (n=24) were exposed to 2-butoxyethanol vapors by inhalation on Gd 6-l 8 at concentrations 0,25,50, 100, or 200 ppm for 6 hours per day (Tyl et al. 1984). The animals were observed for clinical signs throughout the study. Food and water were withheld during exposures. Maternal body weights were taken on Gd 0,6,9, 12, 15, 1821, and 29. The animals were sacrificed on Gd 29 after obtaining blood samples. Hematology and organ weights (uterus, liver, thymus, spleen, and kidney) were evaluated. Hematologic determinations in rabbits indicated no apparent treatment-related changes in any parameter evaluated. Statistically significant increases in hemoglobin content (13.5 g/dL versus 12.4 g/dL in controls) and hematocrit were seen at 100 ppm but not at 200 ppm, suggesting to the authors a biphasic response of the hematological system to 2-butoxyethanol exposure in rabbits Therefore, 100 ppm appears to be a LOAEL and 50 ppm appears to the NOAEL for hematological effects in the rabbits, but the authors did not present a mechanism to explain the apparent biphasic response that these effects were not seen at 200 ppm.

The data indicating that humans are less susceptible to 2-butoxyethanol-induced hemolysis are as follows: In *vitro* experiments designed to examine the effect of 2-butoxyethanol on human and rat erythrocytes (Bartnik et al. 1987), and *in vitro* experiments designed to examine the effect of 2-butoxyacetic acid on the erythrocytes of various mammalian species (including human species) indicated species-dependent sensitivity to the hemolytic effects of 2-butoxyethanol and 2-butoxyacetic acid. In this regard, rodents, rabbits, and baboons were more sensitive than pigs, dogs, cats, guinea pigs, and humans.

In its Threshold Limit Value (TLV) recommendation, tbe American Conference of Governmental Industrial Hygienists (ACGIH) stated that “anemia is not an uncommon condition in the human population. (ACGIH 199 1). ACGIH (199 1) recommended that exposures to 2-butoxyethanol be maintained below levels that are found to cause blood changes in experimental animals. Based on this recommendation, the TLV-TWA was established at 25 ppm.

Agency Contact (Chemical Manager): Olivia Harris

APPENDIX A

Chemical name(s): 2-Butoxyethanol
CAS Number(s): 111-76-2
Date: June 1998
Profile status: Draft 3
Route: [X] Inhalation [ ] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Key to figure: 42
Species: Rat

MRL: 3 [ ] mg/kg/day [X] ppm [ ] mg/m3

Reference: Dodd et al. 1983

Experimental design: Fischer 344 rats (16 male, 16 female) were exposed for 90 days, 6 hours per day, 5 days per week to 2-butoxyethanol target concentrations rof 0,5,25, or 75 ppm (mean average concentrations determined by gas chromatography to be 0,5,25, or 77 ppm, respectively). Rats were observed for signs of toxicity during exposure and 14 days postexposure. Necropsies were performed on animals that died and on survivors killed at the end of the observation period. For bematology evaluation only, 6 rats/sex/group were killed after 6 weeks. Biological assessments included daily animal observations, body weight and organ (kidney, liver, lungs and testes) weight determinations, and ophthalmologic, hematologic, and gross pathologic examinations. In addition, RBC osmotic fragility tests, serum chemistries, urinalysis, and histologic examination of selected tissues of the high BE concentrations were assessed. No deaths occurred throughout the study. There were no clinical signs of toxicity observed. There was a transient decrease in body weight gain (exposure weeks 24; data not shown) of females exposed to 77 ppm 2-butoxyethanol. After 6 weeks (3 1 exposures), female rats exposed to 77 ppm had slight but significant decreases (13% below control values) in red blood cell counts (RBC) and hemoglobin (HGB) accompanied with an 11% increase above control value in mean corpuscular hemoglobin (MCH). At the end of the 90-day study, the hematologic effects seen in the female exposed rats either lessened or returned to control value ranges. The only significant hematologic finding in male rats was a 5% decrease in RBC at 77 ppm after 66 exposures. The red blood cell osmotic fragility tests of both 2-butoxyethanol-treated rats and controls were similar. No treatment-related differences were found in body weight, organ weights, urine or serum chemistries, gross lesions, or microscopic lesions in males or females.

Effects noted in study and corresponding doses:

25 ppm = mid dose (NOAEL)

77 ppm= high dose (Serious LOAEL: female rats had 13% decrease in red blood cells (RBC)
and 4% decrease in hemoglobin [HGB] accompanied with an 11% increase above control value in mean corpuscular bemoglobin [MCH]; male rats had a 5% decrease in RBC)

Dose endpoint used for MRL derivation: 25 ppm - hematological effects

[X] NOAEL [ ] LOAEL

Uncertainty factors used in MRL derivation: 9

[ ] 1 [ ] 3 [ ] 10 (for use of a LOAEL)

[ ] 1 [X] 3 [ ] 10 (for extrapolation from animals to humans)
[ ] 1 [X] 3 [ ] 10 (for human variability)

An uncertainty factor of 3 for animal to human extrapolation was used because *in vitro* data show that human red blood cells are much less sensitive to the hemolytic effects of the 2-butoxyethanol metabolite, 2-butoxy-acetic acid. Concentrations of 2-butoxyacetic acid that caused hemolysis of rat red blood cells did not cause hemolysis of human red blood cells (Bartnik et al. 1987). For example, following a 60 minute exposure of rat red blood cells to 7.5 mM 2-butoxyacetic acid 100% hemolysis was observed. Following a 60 minute exposure of human red blood cells to 15 mM 2-butoxyacetic acid, no hemolysis was observed (Bartnik et al. 1987). An *in vitro* study measuring more sensitive endpoints of red blood cell effects, mean cell volume and hematocrit, has also shown rat red blood cells to be more sensitive to 2-butoxyacetic acid compared to human red blood cells (Ghanayem and Sullivan 1993). For example, following a 1 hour exposure to 2 mM 2-butoxyacetic acid, mean corpuscular volume was about 150% of controls in rat red blood cells, and 105% of controls in human red blood cells, hematocrit was about 155% of controls in rat red blood cells, and 105% of controls in human red blood cells. Red blood cells of rabbits, hamsters, mice and baboons were also sensitive to the red blood cell effects of 2-butoxyacetic acid, while red blood cells of cats, pigs, dogs, and guinea pigs were not sensitive the effects of 2-butoxyacetic acid (Ghanayem and Sullivan 1993). This *in vitro* data is also supported by the Carpenter et al. (1956) study in which erythrocyte osmotic fragility, an effect observed in animals, was not observed in humans exposed by inhalation to ≤ 195 ppm 2-butoxyethanol for 4-8 hours. In addition, an *in vitro* study showed that human erythrocytes were resistant to the hemolytic effects of 2-butoxyacetic acid, the hemolytic metabolite of 2-butoxyethanol (Udden 1994).

An uncertainty factor of 3 for human variability was used because the results of *in vitro* data suggest that 2-butoxyethanol does not cause significant hemolysis of normal and potentially susceptible erythrocytes (Udden 1996). Udden (1994) reported that red blood cells in humans (including the elderly and patients with hereditary spherocytosis and sickle cell disease) were not susceptible to 2-butoxyacetic acid-induced hemolysis.

Was a conversion factor used from uum in food or water to a mg/body weight dose?
If so, explain: Not applicable.

If an inhalation study in animals, list conversion factors used in determining human eauivalent dose:

The human equivalent dose (HEC) was calculated using Formula 4-48 from Interim Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry, EPA (1994). Though 2-butoxyethanol is a Category 2 gas (i.e., water soluble and accumulates in blood), the equation in the EPA (1994) document for extrarespiratory effects of Category 2 gases is presently under review and the recommended equation is that for Category 3 gases:

$$NOAEL_{[HEC]} = NOAEL_{[ADJ]} \times (H_{b/g})_A / (H_{b/g})_H$$

where:

$NOAEL_{[HEC]}$ = the NOAEL human equivalent concentration

$NOAEL_{[ADJ]}$ = the NOAEL adjusted for duration (see below)

$(H_{b/g})_A$ = blood/gas partition coefficient for animals

$(H_{b/g})_H$ = blood/gas partition coefficient for humans

$(H_{b/g})_A$ = not available
$(H_{b/g})_H$ = 7965 (Johanson and Dynesius 1988).

Because a blood/gas partition coefficient was not available for rats, a default of 1 was used for the ratio of partition coefficients.

$NOAEL_{[HEC]}$ = 25 ppm x (1)

$NOAEL_{[HEC]}$ = 25 ppm

Was a conversion used from intermittent to continuous exnosure? If so. exulain: No adjustment for intetmittent exposure was used because 2-butoxyethanol and its major metabolite, 2-butoxyacetic acid, are eliminated from the body within 24 hours. In addition, because of the high blood/gas partition coefficient (7965) relative to a low fat/blood partition coefficient (0.77 Jepson et al. 1992) blood concentrations would be expected to reach periodicity (i.e., the periodic steady state is such that the concentration versus time profile is the same for every week) during intermediate-duration exposure (EPA 1994).

Other additional studies or pertinent information that lend support to this MRL: In addition to the hematologic effects, a transient decrease in body weight was observed in females early in the study. In light of the results of Dodd et al. (1983) after acute inhalation exposure of rats to doses up to 245 ppm, and the more severe hematological effects observed, accompanied by changes in liver weight, respiratory effects, and decreased body weight gain, it seems reasonable to accept the effect seen in the intermediate inhalation exposure experiment as being the most sensitive indicator of toxicity. No other suitable supporting studies were found.

In its Threshold Limit Value (TLV) recommendation, the American Conference of Governmental Industrial Hygienists (ACGIH) stated that “anemia is not an uncommon condition in the human population. (ACGIH 199 1). ACGIH (1991) recommended that exposures to 2-butoxyethanol be maintained below levels that are found to cause blood changes in experimental animals. Based on this recommendation, the TLV-TWA was established at 25 ppm.

Agency Contact (Chemical Manager): Olivia Harris

APPENDIX A

Chemical Name: 2-Butoxyethanol
CAS Number: 111-76-2
Date: June 1998
Profile Status: Draft 3
Route: [x] Inhalation [ ] Oral
Duration: [] Acute [] Intermediate [x] Chronic
Graph Key: 51
Species: Human

MRL: 0.2 [ ] mg/kg/day [x] ppm

Reference: Haufroid et al. 1997

Experimental design:

Thirty-one male workers exposed to 2-butoxyethanol were studied. Twenty workers were exposed to an average concentration of 0.75 ppm, and 11 workers were exposed to an average concentration of 0.46 ppm. The weighted average of the means for the two groups of workers is 0.6 ppm. The workers were exposed for 1 to 6 years. The workers were also exposed to methyl ethyl ketone. Exposure concentrations of methyl ethyl ketone were not provided. Studies in animals indicate that methyl ethyl ketone does not produce hematologic effects (ATSDR 1992). The average urinary concentrations of methyl ethyl ketone were 0.27 mg/g creatinine. Twenty-one unexposed men that worked for the same company served as controls; however, 2-butoxyethanol concentrations in the air the controls breathed were not assayed. Urine was collected before the shift and at the end of the shift and assayed for free 2-butoxyacetic acid (thought to be the toxic metabolite), retinol binding protein, and creatinine. Rettenmeier et al. (1993) and Sakai et al. (1994) found that some of the 2-butoxyacetic acid (BAA) in the urine of humans is excreted as amino acid conjugates . Rettenmeier et al. (1993) reported that the ratio of the amino acid conjugate of butoxyacetic acid to total butoxyacetic acid ranged from 0.16 to 0.64 (mean value 0.48). Thus, free BAA in urine does not represent all internal dosing. Blood was collected and assayed for red blood cell counts, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, haptoglobin, reticulocytes, and osmotic resistance. Serum alanine aminotransferase and aspartate aminotransferase were also measured. The exposed workers wore gloves, thus minimizing dermal exposure. In addition, two exposure groups were combined together for the analysis of measured parameters.

Effects noted in study and corresponding doses:

Urinary concentrations of free 2-butoxyacetic acid ranged from not detected to 1.1 mg/g creatinine before shift (average 0.3), and 0.3 to 51.4 mg/g creatinine after shift (average 12.2) in persons exposed to an average of 0.76 ppm. Urinary concentrations of free 2-butoxyacetic acid ranged from not detected to 3.4 mg/g creatinine before shift (average 0.6), and 0.6 to 20.4 mg/g creatinine after shift (average 9.2) in persons exposed to an average of 0.46 ppm. A significant correlation ($r = 0.55$, $p = 0.0012$) was observed between endshift urinary free 2-butoxyacetic acid concentrations and 2-butoxyethanol in air. There was no effect on red blood cell numeration, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, haptoglobin, reticulocyte numeration, osmotic resistance, hepatic (aspartate aminotransferase, alanine aminotransferase) and renal (plasmatic creatinine, urinary retinol binding protein) parameters. Two small but statistically significant differences in hematology values were observed: a significant decrease ($p=0.03$) in hematocrit values (exposed: 43.9% ± 2.1, range: 39.9-50.7, controls: 45.5% ± 2.7, range: 40.6-50.4) and a significant ($p=0.02$) increase in mean corpuscular hemoglobin concentration (exposed: 33.6 g/dL ± 0.9, range: 31.8-35.6; controls: 32.9 g/dL ±1.1, range: 31.1-35.6). ATSDR considers these differences to be consistent with hemolysis observed in animal studies, and they may be early indicators of potential adverse effects in humans. However,

because the changes in both hematocrit and mean corpuscular hemoglobin concentration were in the range of normal clinical values, the effect was considered a NOAEL. Normal clinical values of hematocrit are reported for males as 40-54%, with a level of 30% or less considered to indicate moderate to severe anemia (Fischbach 1992). Normal clinical values of mean corpuscular hemoglobin concentration are reported as 3 l-37 g hemoglobin/dL (Fischbach 1992). Increased mean corpuscular hemoglobin concentrations often indicates spherocytosis, while decreased mean corpuscular hemoglobin concentrations may indicate macrocytic anemia, chronic blood loss anemia, or pyridoxine-responsive anemia (Fischbach 1992). None of the red blood cell end points were correlated with internal exposure as assessed by urinary free 2-butoxyacetic acid. [Although as noted above a significant fraction of BAA in urine may have been conjugated.] Serum creatinine and urinary retinol binding protein were not affected. No difference was observed for serum alanine transaminase or serum aspartate transaminase. The statistics in this paper seem odd.

Dose and end point used for MRL derivation:

[X] NOAEL [] LOAEL

0.6 ppm

Uncertainty Factors used in MRL derivation: 3

[x] 1 [ ] 3 [ ] 10 (for use of a LOAEL)
[x] 1 [ ] 3 [ ] 10 (for extrapolation from animals to humans)
[ ] 1 [x] 3 [ ] 10 for human variability

An uncertainty factor of 3 for human variability was used because the results of *in vitro* data suggest that 2-butoxyethanol does not cause significant hemolysis of normal and potentially susceptible erythrocytes (Udden 1996). Udden (1994) reported that red blood cells in humans (including the elderly and patients with hereditary spherocytosis and sickle cell disease) were not susceptible to 2-butoxyacetic acid-induced hemolysis. At the end of a 90-day inhalation study, the hematologic effects seen in the female exposed rats either lessened or returned to control value ranges (Dodd et al 1983).

Was a conversion used from ppm in food or water to a mg/body weight dose?
If so, explain: Not applicable

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose: Not applicable

Other additional studies or pertinent information which lend suuuort to this MRL:

There are no additional chronic studies in either humans or animals.

Agency Contact (Chemical Manager): Olivia Harris

APPENDIX A

Chemical name(s): 2-Butoxyethanol
CAS Number(s): 111-76-2
Date: June 1998
Profile status: Draft 3
Route: [ ] Inhalation [X] Oral
Duration: [X] Acute [ ] Intermediate [ ] Chronic
Key to figure: 28
Species: Rat

MRL: 0.4 [X] mg/kg/day [ ] ppm [ ] mg/m3

Reference: Ghanayem et al. 1987a

Experimental design: Young (4-5 weeks) and adult (9-13 weeks, 5-6 months, and 16 months) rats were dosed with 0,32,63, 125,250, or 500 mg/kg 2-butoxyethanol by gavage. Hemotoxicity and metabolism and excretion of 2-butoxyethanol was monitored for 48 hours. Animals were killed at 24 and 48 hours after treatment and spleen, liver, kidney, testes, and urinary bladder were weighed, fixed, and examined. Histopathological results were presented for the control, 125,250, and 500 mg/kg dose group.

Effects noted in study and corresuonding doses: In younger rats, 125 mg/kg caused no effect on hematological parameters. At 500 mg/kg, RBC, HGB, and HCT were decreased, although not as much as in the older rats. In older rats, 2-butoxyethanol at 125 mg/kg causes severe acute hemolytic anemia resulting in significant decreases at 8 and 24 hours after treatment in RBCs, HGB, and HCT, and increases 8 hours after administration in the concentration of free plasma hemogliobin. At 4 hours, peak increases in free plasma hemoglobin were seen in both younger and older rats dosed with 500 mg/kg. Secondary to the hemolytic effects, 2-butoxyethanol also caused hemoglobinuria in 16-month-old rats at ≥32 mg/kg, in 6-month-old rats at ≥63 mg/kg, and in younger rats (4-13 weeks) at ≥125 mg/kg. Other effects secondary to hemolysis consisted of histopathologic changes in the liver and kidney, including focal coagulative necrosis of hepatocytes at 250 mg/kg (l/6) and hemoglobin casts in the proximal tubules of the kidney at 125 mg/kg in adult (9-13 weeks) rats; these effects were not seen in young (4-5 weeks) rats even at 500 mg/kg. These effects of 2-butoxyethanol were dose- and time-dependent, and were more severe at 24 hours after treatment that at 48 hours after treatment. The onset of the development of hemoglobinuria followed the decline in the concentration of free HGB in the plasma. Both the hemolytic effects and the secondary effects of 2-butoxyethanol were age-dependent, with older rats being more sensitive than younger rats. The 4-5-week-old rats and 9-13-week-old rats were treated with 500 mg/kg/day 2-butoxyethanol, and reticulocytes and leukocytes were counted up to 48 hours after treatment. Adult rats exhibited neutrophilic leukocytosis and mild lymphopenia within 4 hours. This was not detected at 48 hours. An absolute increase in reticulocyte count occurred at 48 hours after dosing. Thus,

32 mg/kg = LOAEL.

Dose endpoint used for MRL derivation: 32 mg/kg - hematological effects

[ ] NOAEL [X] LOA L

Uncertainty factors used in MRL derivation: 90

[ ] 1 [ ] 3 [X] 10 (for use of a LOAEL)
[ ] 1 [X] 3 [ ] 10 (for extrapolation from animals to humans)
[ ] 1 [X] 3 [ ] 10 (for human variability)

Since the 32 mg/kg dose in the Ghanayem et al. (1987a) study caused hemoglobinuria only in the aged rats, the uncertainty factor used for human variability was 3 since the LOAEL is for the most sensitive population. Also, *in vitro* data show that human red blood cells are much less sensitive to the hemolytic effects of the 2-butoxyethanol metabolite, 2-butoxyacetic acid. Concentrations of 2-butoxyacetic acid that caused hemolysis of rat red blood cells did not cause hemolysis of human red blood cells (Bartnik et al. 1987). For example, following a 60 minute exposure of rat red blood cells to 7.5 mM 2-butoxyacetic acid 100% hemolysis was observed. Following a 60 minute exposure of human red blood cells to 15 mM 2-butoxyacetic acid, no hemolysis was observed (Bartnik et al. 1987). An *in vitro* study measuring more sensitive endpoints of red blood cell effects, mean cell volume and hematocrit, has also shown rat red blood cells to be more sensitive to 2-butoxyacetic acid compared to human red blood cells (Ghanayem and Sullivan 1993). For example, following a 1 hour exposure to 2 mM 2-butoxyacetic acid, mean corpuscular volume was about 150% of controls in rat red blood cells, and 105% of controls in human red blood cells, hematocrit was about 155% of controls in rat red blood cells, and 105% of controls in human red blood cells. Red blood cells of rabbits, hamsters, mice and baboons were also sensitive to the red blood cell effects of 2-butoxyacetic acid, while red blood cells of cats, pigs, dogs, and guinea pigs were not sensitive the effects of 2-butoxyacetic acid (Ghanayem and Sullivan 1993). This *in vitro* data is also supported by the Carpenter et al. (1956) study in which erythrocyte osmotic fragility, an effect observed in animals, was not observed in humans exposed by inhalation to ≤195 ppm 2-butoxyethanol for 4-8 hours.

An uncertainty factor of 3 was used for human variability because the LOAEL is from a sensitive subpopulation (older animals). Also, an uncertainty factor of 3 for human variability was used because the results of *in vitro* data suggest that 2-butoxyethanol does not cause significant hemolysis of normal and potentially susceptible erythrocytes (Udden 1996). Udden (1994) reported that red blood cells in humans (including the elderly and patients with hereditary spherocytosis and sickle cell disease) were not susceptible to 2-butoxyacetic acid-induced hemolysis.

Was a conversion factor used from ppm in food or water to a mg/body weight dose? If so, explain:
Not applicable.

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.
Was a conversion used from intermittent to continuous exposure?
If so, explain: Not applicable

Other additional studies or pertinent information that lend support to this MRL: Since the severity of hemoglobinuria in the 16-month-old rats increased as the dose levels increased and since hemoglobinuria occurred in younger rats only at higher doses (Ghanayem et al. 1987a), the 32 mg/kg dose was considered to be a less serious LOAEL. The use of the 32 mg/kg as a Less serious LOAEL for the derivation of the acute oral MRL is supported by a study in pregnant rats, in which no hematological effects were found at a similar dose of 30 mg/kg/day given for 3 days during gestation (NTP 1989) (see discussion below). Since the 32 mg/kg dose in the Ghanayem et al. (1987a) study caused hemoglobinuria only in the aged rats, the uncertainty factor used for human variability was 3, since the LOAEL is for the most sensitive population. Furthermore, since humans appear to be less susceptible to 2-butoxyethanol-induced hemolysis (see below), the uncertainty factor used for extrapolation from animals to humans was 3.

Hemolytic anemia is the characteristic toxic reaction to 2-butoxyethanol in animals. Sperm-positive Fischer 344 rats (27-33 per dose group) were administered 0,30, 100, and 200 mg/kg/day 2-butoxyethanol (BE) in distilled water by gavage for Gd 9-11 (NTP 1989). The biological effects of BE on dams were assessed during treatment as well as 24 hours after treatment and on Gd 20, and on fetuses at sacrifice

(Gd 20); 22-24 females per group were confirmed pregnant. At sacrifice on Gd 12 or 20, each pregnant female was examined by counting number of corpora Butea, and weighing body, liver, right and left kidney, spleen and uterus. For females sacrificed on Gd 20, the uterine contents were also evaluated (i.e., number of implantation sites, resorptions, and dead and live fetuses). The fetuses were sexed and examined for external and visceral malformations. Maternal and fetal blood (pooled by litter) was analyzed for red (BBC) and white (WBC) blood cell counts, hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and platelet count. RBC count and corresponding measures of HCT and HGB were significantly reduced in the 100 and 200 mg/kg/day dose groups at approximately 24 hours after the final dose. MCHC was significantly decreased at the high dose group. The blood dyscrasia produced at approximately 24 hours after the last treatment also included increases in reticulocytes (%RBC), WBC count, platelet count, MCV, and MCH in the 100 and 200 mg/kg/day dose groups. On Gd 20, maternal hematological end points recovered toward control values, although a majority of values remained different from controls on the 100 and 200 mg/kg/day dose groups RBC counts measured on Gd 20 in the 100 and 200 mg/kg/day dose groups of each treatment period were still significantly lower than control values, but counts had increased since cessation of treatment (i.e., at 200 mg/kg/day dose group), the RBC count increased to 9 1% of control levels by Gd 20. Similarly, reticulocytes declined from values measured earlier in the 100 and 200 mg/kg/day dose groups, but were still significantly above the control level. Corrected WBC count measured on Gd 20 after dam treatment was similar across dose groups. Platelet counts determined at the end of the study after treatment showed a significant decreasing trend with dose which appeared to be determined by a nonsignificant decreasing trend with the high-dose group below the control count. The MCV and MCH on Gd 20 exhibited significant dose-related trends with the values of the two highest dose groups significantly greater than the control values. Calculated values of MCHC showed significant decreasing dose-related trends with the high-dose group values lower than controls. The changes in MCHC with treatment, although significant at the high-dose group, were relatively slight. The maternal hematological profiles at 24 hours after final treatment and at Gd 20 indicate that 2-butoxyethanol-induced hemolysis triggered a compensatory hematopoietic response leading to reticulocytosis. Furthermore, recovery was more complete by Gd 20 for dams treated on Gd 9-l 1 than those exposed to Gd 1 l-l 3.

In a similar experiment by NTP (1989), sperm-positive female Fischer 344 rats (29-31 per dose group) were administered 0,30, 100, and 300 mg/kg/day 2-butoxyethanol in distilled water by gavage for Gd 1 l-13. The biological effects of BE on dams were assessed during treatment as well as 24 hours after treatment and on Gd 20, and on fetuses at sacrifice (Gd 20). Maternal and fetal blood (pooled by litter) was analyzed for red (BBC) and white (WBC) blood cell counts, hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and platelet count. RBC count and corresponding measures of HCT and HGB were significantly reduced in the 100 and 300 mg/kg/day dose groups at approximately 24 hours after the final dose. MCHC was significantly decreased at the high dose group. The blood dyscrasia produced at approximately 24 hours after the last treatment also included increases in reticulocytes (%RBC), WBC count, platelet count, MCV, and MCH in the 100 and 300 mg/kg/day dose groups. On Gd 20, maternal hematological end points recovered toward control values, although a majority of values remained different from controls on the 100 and 300 mg/kg/day dose groups. RBC counts measured on Gd 20 in the 100 and 300 mg/kg/day dose groups of each treatment period were still lower than control values, but counts had increased since cessation of treatment (i.e., at 300 mg/kg/day dose group), the RBC count increased from about 45% of control levels at 24 hours after the final treatment to 80% of control levels by Gd 20. Similarly, reticulocytes declined from values measured earlier in the 100 and 300 mg/kg/day dose groups, but were still significantly above the control level. Corrected WBC count measured on Gd 20 after dam treatment still exhibited a significant increasing trend with dose which appeared to be determined by a nonsignificant increase in the high-dose group. Platelet counts determined at the end of the study after treatment showed a significant increase at the high dose. The MCV and MCH on Gd 20 exhibited significant dose-related trends with the values of the two highest dose

groups greater than the control values. Calculated values of MCHC showed significant decreasing dose-related trends with the high-dose group values lower than controls. The changes in MCHC with treatment, although significant at the high-dose group, were relatively slight. The maternal hematological profiles at 24 hours after final treatment and at Gd 20 indicate that BE-induced hemolysis triggered a compensatory hematopoietic response leading to reticulocytosis. Furthermore, recovery was more complete by Gd 20 for dams treated on Gd 9-l 1 than those exposed to Gd 11-13. Thus, 30 mg/kg/day was the NOAEL for hematological effects in both experiments in the NTP (1989) study, lending support for the use of the LOAEL of 32 mg/kg for hematological effects in the aged rats in the Ghanayem et al. (1987a) study for the derivation of the acute oral MRL.

In another study (Ghanayem et al. 1992) male Fischer 344 rats (n=6) received by gavage 125 mg/kg/day 2-butoxyethanol for 1,2, 3,6, or 12 consecutive days. Controls received 5 mL water/kg body weight daily for 12 consecutive days. Twenty-four hours after tbe last dose, blood samples were collected for hematological measurements and determination of blood ATP concentration. Spleen and liver were removed and weighed. Organ weight per 100 g body weight ratios were calculated. Treatment of rats with 2-butoxyethanol daily (125 mg/kg/day) for l-3 consecutive days resulted in a time-dependent increase in the hemolysis of erythrocytes. However, when daily treatment with 2-butoxyethanol continued beyond 3 days, the number of erythrocytes began to rebound and approached pretreatment levels within 12 days despite continued daily exposure, suggesting development of tolerance to the hemolytic effect of 2-butoxyethanol. Mean cell volumes (MCV), ATP concentration, and the number of reticulocytes increased to the sixth day and then decreased on the twelfth day. Liver weight/body weight ratios were minimally affected by repeated dosing, declined on days 3 and 6, but increased on day 12 as compared to controls. Spleen weight increased in a time-dependent manner.

In other studies, hematotoxic effects were observed at similar or higher doses. For instance, hemoglobinuria was observed in rats receiving one dose of 126 mg/kg (Corley et al. 1994); hemolysis was observed in rats receiving one dose of 250 mg/kg, and reduced BBC, hematocrit, and increased MCV and reticulocytes, and mean corpuscular hemoglobin in the presence of increased liver and spleen weight were observed in rats at 500 mg/kg/day after 1 or 4 days (Ghanayem and Sullivan 1993; Ghanayem et al. 1987b; Grant et al. 1985). Hepatic and renal histopathology similar to that observed in Ghanayem et al. (1987a) at 125 mg/kg/day was also observed at 500 mg/kg/day after one dose (Ghanayem et al. 1987b).

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APPENDIX A

Chemical name(s) : 2-Butoxyethanol
CAS Number(s): 111-76-2
Date: June 1998
Profile status: Draft 3
Route: [ ] Inhalation [X] Oral
Duration: [ ] Acute [X] Intermediate [ ] Chronic
Key to figure: 90
Species: Rat

MRL: 0.07 [X] mg/kg/day [ ] ppm [ ] mg/m^3

Reference: NTP 1993

Experimental design: F344/N rats (10 males, 10 females) were given targeted concentrations of 0,750, 1,500,3,000,4500, or 6,000 ppm 2-butoxyethanol in drinking water daily for 13 weeks. Actual doses, determined by the authors from drinking water consumption and body weight data, were: 0,69, 129,281, 367, and 452 mg/kg/day for males and 0, 82, 151, 304, 363, and 470 mg/kg/day for females. Animals were observed twice daily and weighed at the start of the studies, weekly thereafter, and at necropsy. Clinical observations were recorded weekly. Water consumption by cage was measured twice a week. Complete necropsies were performed on all animals in the base studies. The heart, liver, right kidney, lung, thymus, and right testis were weighed, examined for gross lesions, fixed or embedded in paraffin, and stained for microscopic examination. The protocol for the 13-week studies required that tissues be examined microscopically in all control animals, animals in the highest dose group with at least 60% survivors, and all animals in the higher-dose group (inclusive of early deaths and survivors). In the clinical pathology study, 20 males and 20 females per dose group were used. On days 5 and 21, blood samples were collected for the clinical pathology and hematology studies. Week- 13 analyses were conducted on samples obtained from rats in the base studies. Urinalysis was done on week- 13 samples collected overnight from the base study animals. In the sperm morphology and vaginal cytology evaluations, males were evaluated for necropsy body and reproductive tissue weights, and spermatozoa1 data. Females were evaluated for necropsy body weight, estrous cycle length, and percentage of cycle spent in the various stages.

Effects noted in study and corresponding doses: Hematological effects were noted at ≥ 82 mg/kg/day in females and at 281 mg/kg/day in males, but no hematological effects were found in males at 69 or 129 mg/kg/day. Females were more sensitive than males to the hematological effects. Hepatic effects were also noted at ≥ 82 mg/kg/day in females and ≥ 69 mg/kg/day for males. The hepatic effects included significantly increased levels of serum alkaline phosphatase in high dose males (452 mg/kg/day) at 3 weeks and in females at 363 and 470 mg/kg/day at 13 weeks, and in serum alanine aminotransferase in males at 452 mg/kg/day and in females at 363 and 470 mg/kg/day at week 13. Histological examination of livers revealed hepatocellular alterations (hepatocytes that stained more eosinophilic and lacked the amphophilic to basophilc granularity of the cytoplasm) at all doses (≥ 69 mg/kg/day for males and ≥ 82 mg/kg/day for females). The changes may be indications of early degeneration changes in hepatocytes. Centrilobular hepatocellular degeneration was observed in males at ≥ 281 mg/kg/day and in females at ≥ 304 mg/kg/day; and brown to green granular pigment staining strongly positive for iron in Kupffer's cell cytoplasm in males at 452 mg/kg/day and in females at ≥ 151 mg/kg/day. Renal effects were also seen in males and females. Renal effects included moderate increases in blood urea nitrogen in males at ≥ 69 mg/kg/day at week 3 and at ≥ 281 mg/kg/day at week 13 and in females at ≥ 304 mg/kg/day at week 13; increases in blood creatinine in females at ≥304 mg/kg/day at week 13; decreases in total blood protein in males at ≥281 mg/kg/day at week 13 and in females at ≥ 151 mg/kg/day at weeks 3 and 13; decreases in blood albumin in males at ≥281 mg/kg/day at week 13 and in females at ≥ 363 mglkg/day at week 3 and at ≥304 mg/kg/day at week 13; decreased urine volume in females at ≥ 82 mg/kg/day at week 13; and increased specific gravity of the urine in

males at ≥ 69 mg/kg/day and in females at ≥151 mg/kg/day at week 13. However, histological examination of the kidneys and urinary bladder revealed no pathological lesions. The renal effects observed in rats may have reflected dehydration, since drinking water consumption was decreased. Thus, the 69 mg/kg/day dose in male rats is a less serious LOAEL for both hepatic and renal effects, and therefore is an appropriate dose for the derivation of the intermediate oral MRL.

69 mg/kg/day = low dose in males (LOAEL for hepatic and renal effects) - hepatocellular alteration, moderate increased blood urea nitrogen.

82 mg/kg/day = low dose in females - decreased RBC, HCT, HGB; hepatocellular alteration; decreased urine volume.

Dose endpoint used for MRL derivation: 69 mg/kg/day - hepatic effects

[ ] NOAEL [X] LOAEL

Uncertainty factors used in MRL derivation: 1000

[ ] 1 [ ] 3 [X] 10 (for use of a LOAEL)
[ ] 1 [ ] 3 [X] 10 (for extrapolation from animals to humans)
[ ] 1 [ ] 3 [X] 10 (for human variability)

Was a conversion factor used from ppm in food or water to a mg/body weight dose?
If so, explain: Doses in mg/kg/day were calculated by NTP (1993) from drinking water consumption and body weight data in the experimental animals

If an inhalation study in animals, list conversion factors used in determining human equivalent dose: Not applicable.

Was a conversion used from intermittent to continuous exposure? If so. exulain: Not applicable.

Other additional studies or pertinent information that lend support to this MRL:

In the same study by NTP (1993), groups of 10 male and 10 female B6C3F$_1$ mice were given targeted concentrations of 0,750, 1,500,3,000,4500, or 6,000 ppm 2-butoxyethanol in drinking water daily for 13 weeks. Actual doses were: 0, 118,223,553,676, and 694 mg/kg/day for males and 0, 185,370,676, 86 1, and 1,306 mg/kg/day for females. Animals were observed twice daily and weighed at the start of the studies, weekly thereafter, and at necropsy. Clinical observations were recorded weekly. Water consumption by cage was measured twice a week. Complete necropsies were performed on all animals in the base studies. The heart, right kidney, lung, thymus, and right testis were weighed, examined for gross lesions, fixed or embedded in paraffin, and stained for microscopic examination" The protocol for the 13-week studies required that tissues be examined microscopically in all control animals, animals in the highest dose group with at least 60% survivors, and all animals in the higher-dose group (inclusive of early deaths and survivors). In the sperm morphology and vaginal cytology evaluations, males were evaluated for necropsy body and reproductive tissue weights, and spermatozoa1 data. Females were evaluated for necropsy body weight, estrous cycle length, and percentage of cycle spent in the various stages. Clinical chemistry, urinalysis, and hematological evaluations were not performed for the mice. The only effect observed in mice was decreased body weight gain in male mice at t ≥53 mg/kg/day and in female mice at ≥ 676 mg/kg/day. No hepatic or renal effects were found in the mice at ≤ 694 mg/kg/day (males) and ≤ 1,306 mg/kg/day (females).

The only other comprehensive intermediate oral study was conducted in adult male albino rats (COBS CD (SD)BR) that were given undiluted 2-butoxyethanol by gavage in doses of 0,222,443, or 885 mg/kg/day, 5 days per week over a 6-week period (Eastman Kodak 1983; Krasavage 1986). Body weights and feed consumption were recorded. Clinical signs of toxicity and deviations from normal appearance and behavior were observed. Mortality was checked daily and dead animals were necropsied; tissues (lung, heart, thymus, kidneys, liver, spleen, brain, salivary glands, stomach, cecum, colon, duodenum, jejunum, ileum, pancreas, esophagus, adrenal glands, pituitary, thyroid, parathyroid, trachea, mesenteric lymph nodes, testes, epididymides, prostate, seminal vehicles, coagulating gland, bone marrow, tongue, and nasal cavities) were collected for histologic examination. Eyes were fixed in tinker's solution. Organ weights of liver, kidneys, heart, testes, brain, and spleen were determined. The LOAEL (serious) for hematological effects was 222 mg/kg/day, and there was no NOAEL for that effect Hepatic and renal effects associated with the hemolytic effects occurred at 443 mg/kg/day (less serious LOAEL), but not at 222 mg/kg/day, and included hemosiderin disposition and an increase in alkaline phosphatase activity.

Agency Contact (Chemical Manager): Olivia Harris

APPENDIX B

USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Tables and Figures for Levels of Significant Exposure (LSE)

Tables (2-l) 2-2, and 2-3) and figures (2- 1 and 2-2) are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, minimal risk levels (MRLs) to humans for noncancer end points, and EPA's estimated range associated with an upper- bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of No-Observed-Adverse-Effect Levels (NOAELs), Lowest-Observed-Adverse-Effect Levels (LOAELs), or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 2-l and Figure 2-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See LSE Table 2-l

(1) Route of Exposure One of the first considerations when reviewing the toxicity of a subs_tance using these tables and figures should be the relevant and appropriate route of exposure. When sufficient data exists, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Table 2- 1, 2-2, and 2-3, respectively). LSE figures are limited to the inhalation (LSE Figure 2-l) and oral (LSE Figure 2-2) routes. Not all substances will have data on each route of exposure and will not therefore have all five of the tables and figures.

(2) Exposure Period Three exposure periods - acute (less than 15 days), intermediate (15-364 days), and chronic (365 days or more) are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects

occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the 2 "18r" data points in Figure 2-l).

(5) Species The test species, whether animal or human, are identified in this column. Section 2.5, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 2.3, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAHLs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration The duration of the study and the weekly and daily exposure regimen are provided in this column This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to 1 ,1 ,2,2-tetrachloroethane via inhalation for 6 hours per day, 5 days per week, for 3 weeks. For a more complete review of the dosing regimen refer to the appropriate sections of the text or the original reference paper, i.e., Nitschke et al. 198 1.

(7) System This column further defines the systemic effects These systems include: respiratory, cardiovascular, gastrointestinal, hematological, muscmoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, 1 systemic effect (respiratory) was investigated.

(8) NOAEL A No-Observed-Adverse-Effect Level (NOAEL) is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for the respiratory system which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL A Lowest-Observed-Adverse-Effect Level (LOAEL) is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELS.

(10) Reference The complete reference citation is given in chapter 8 of the profile.

(11) CEL A Cancer Effect Level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Figure 2-1

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period The same exposure periods appear as in the LSE table. In this example, health effects observed within the intermediate and chronic exposure periods are illustrated.

(14) Health Effect These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/ m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL In this example, 18r NOAEL is the critical endpoint for which an intermediate inhalation exposure MBL is based. As you can see from the LSE figure key, the open-circle symbol indicates to a NOAEL for the test species-rat" The key number B 8 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the Table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL Key number 38r is 1 of 3 studies for which Cancer Effect Levels were derived. The diamond symbol refers to a Cancer Effect Level for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,OOO,OOO. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q1*).

(19) Key to LSE Figure The Key explains tRe abbreviations and symbols used in the figure.

1 →

TABLE 2-1. Levels of Significant Exposure to [Chemical x] – Inhalation

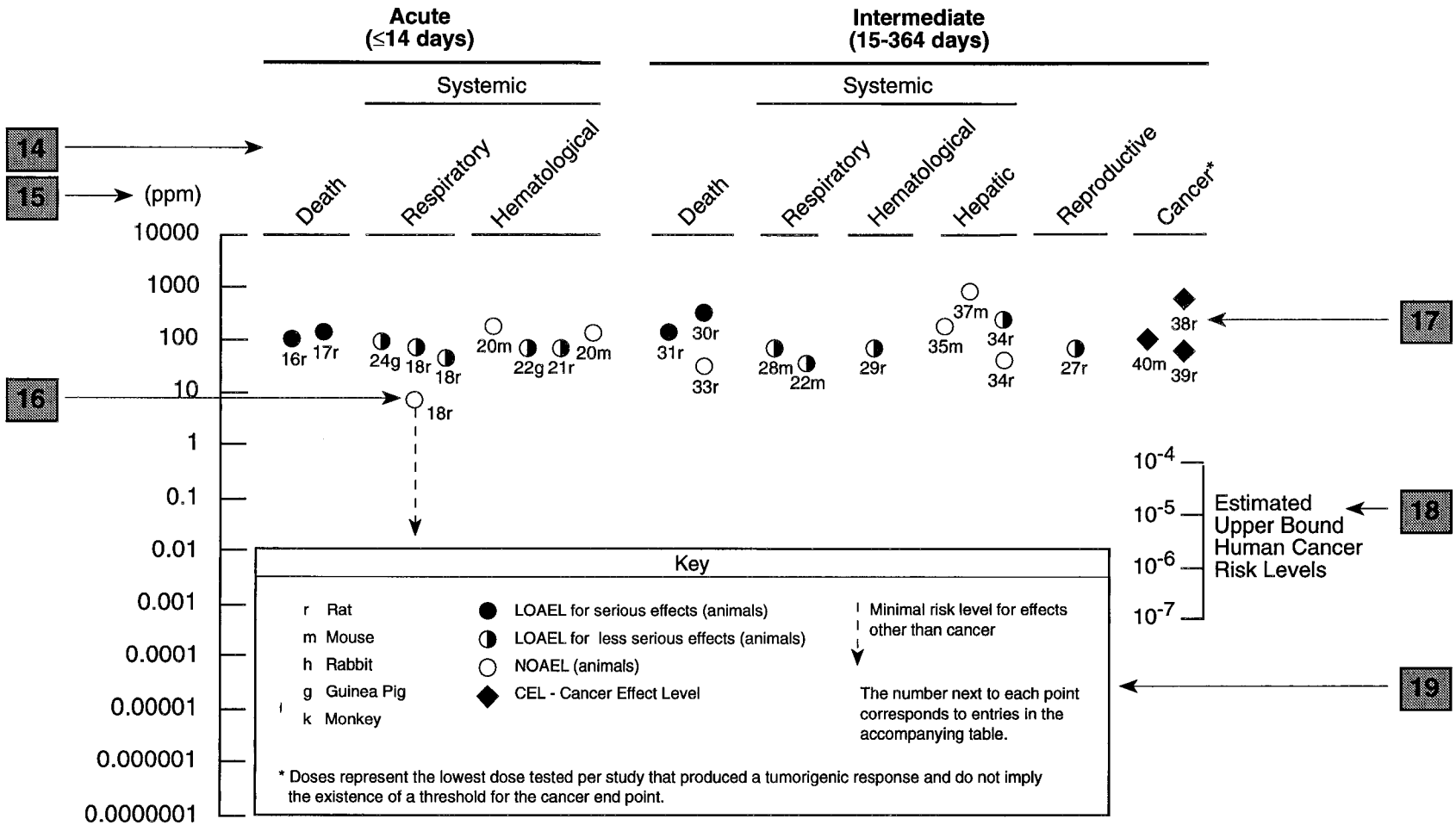
Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect) Less serious (ppm)	Reference
2 → INTERMEDIATE EXPOSURE						
	5	6	7	8	9	10
3 → Systemic	↓	↓	↓	↓	↓	↓
4 → 18	Rat	13 wk 5d/wk 6hr/d	Resp	3 [b]	10 (hyperplasia)	Nitschke et al. 1981
CHRONIC EXPOSURE						
					11	
Cancer					↓	
38	Rat	18 mo 5d/wk 7hr/d			20 (CEL, multiple organs)	Wong et al. 1982
39	Rat	89–104 wk 5d/wk 6hr/d			10 (CEL, lung tumors, nasal tumors)	NTP 1982
40	Mouse	79–103 wk 5d/wk 6hr/d			10 (CEL, lung tumors, hemangiosarcomas)	NTP 1982

[a] The number corresponds to entries in Figure 2-1.

12 → [b] an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

SAMPLE

13 → **Figure 2-1. Levels of Significant Exposure to [Chemical X] – Inhalation**



Chapter 2 (Section 2.5)

Relevance to Public Health

The Relevance to Public Health section provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions.

1 . What effects are known to occur in humans?

2 . What effects observed in animals are likely to be of concern to humans?

3 . What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The section covers end points in the same order they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this section. If data are located in the scientific literature, a table of genotoxicity information is included.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal risk levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Data Needs section.

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, we have derived minimal risk levels (MRLs) for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action; but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans. They should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2.5, “Relevance to Public Health,” contains basic information known about the substance. Other sections such as 2.7, “Interactions with Other Substances,” and 2.8, “Populations that are Unusually Susceptible” provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses for lifetime exposure (RfDs).

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot

make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest NOAEL that does not exceed any adverse effect levels. When a NOAEL is not available, a Lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the LSE Tables.

APPENDIX C

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ADME	Absorption, Distribution, Metabolism, and Excretion
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
BSC	Board of Scientific Counselors
C	Centigrade
CDC	Centers for Disease Control
CEL	Cancer Effect Level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
CLP	Contract Laboratory Program
cm	centimeter
CNS	central nervous system
d	day
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DOL	Department of Labor
ECG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
EKG	see ECG
F	Fahrenheit
F_1	first filial generation
FAO	Food and Agricultural Organization of the United Nations
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
fpm	feet per minute
ft	foot
FR	*Federal Register*
g	gram
GC	gas chromatography
gen	generation
HPLC	high-performance liquid chromatography
hr	hour
IDLH	Immediately Dangerous to Life and Health
IARC	International Agency for Research on Cancer
ILO	International Labor Organization
in	inch
Kd	adsorption ratio
kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient

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L	liter
LC	liquid chromatography
LC_{Lo}	lethal concentration, low
LC_{50}	lethal concentration, 50% kill
LD_{Lo}	lethal dose, low
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
m	meter
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mm Hg	millimeters of mercury
mmol	millimole
mo	month
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
ng	nanogram
nm	nanometer
NHANES	National Health and Nutrition Examination Survey
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPL	National Priorities List
NRC	National Research Council
NTIS	National Technical Information Service
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration
PEL	permissible exposure limit
pg	picogram
pmol	picomole
PHS	Public Health Service
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure limit
RfD	Reference Dose
RTECS	Registry of Toxic Effects of Chemical Substances
sec	second
SCE	sister chromatid exchange
SIC	Standard Industrial Classification
SMR	standard mortality ratio
STEL	short term exposure limit

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STORET	STORAGE and RETRIEVAL
TLV	threshold limit value
TSCA	Toxic Substances Control Act
TRI	Toxics Release Inventory
TWA	time-weighted average
U.S.	United States
UF	uncertainty factor
yr	year
WHO	World Health Organization
wk	week
>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
δ	delta
γ	gamma
μm	micrometer
μg	microgram