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LETTER TRANSMITTING MEMORANDUM IN RESPONSE TO REQUEST FOR INHALATION
AND ORAL TOXICITY INFORMATION FOR BENZENE CNC CHARLESTON SC
8/23/1994
U S EPA REGION IV



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND DEVELOPMENT
ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE
CINCINNATI, OHIO 45268

MEMORANDUM

DATE: August 23, 1994

SUBJECT: Chronic Toxicity Information for Benzene (CASRN 71-43-2) for the Calhoun Park/Ansonborough Homes Site/Charleston, SC.

FROM: Joan S. Dollarhide *Joan Dollarhide*
Director
Superfund Health Risk Technical Support Center
Chemical Mixtures Assessment Branch

TO: Glenn Adams
U.S. EPA
Region IV

This memorandum responds to your request for inhalation and oral toxicity information for benzene (CASRN 71-43-2) for the Calhoun Park/Ansonborough Homes Site in Charleston, SC.

Please find attached the following Risk Assessment Issue Papers:

- Attachment I. Risk Assessment Issue Paper for: Derivation of a Provisional Oral RfD for Benzene (CASRN 71-43-2)
- Attachment II. Risk Assessment Issue Paper for: Derivation of a Provisional Chronic Inhalation RfC for Benzene (CASRN 71-43-2)

Please contact the Superfund Health Risk Technical Support Center at (513) 569-7300 with any additional questions.

cc: E. Akin (Region IV RTIC)



**Risk Assessment Issue Paper for:
Derivation of a Provisional Oral RfD for Benzene (CASRN 71-43-2)**

INTRODUCTION

No chronic RfD or RfC is available on IRIS (U.S. EPA, 1994) or HEAST (U.S. EPA, 1993a). Documents listed on the CARA list (U.S. EPA, 1993b) include an AWQCD (U.S. EPA, 1980, 1989a) and HEA (U.S. EPA, 1984, 1989b). None of these documents derived non-carcinogenic estimates of risk from benzene exposure. The Drinking Water Regulations and Health Advisories list indicates an MCLG of zero, an MCL of 0.005, and 1- and 10-day health advisories (10-kg child) of 0.2 mg/L (U.S. EPA, 1993d); the 1- and 10-day health advisories were derived from an inhalation study (U.S. EPA, 1993c). Longer-term health advisories for a child and/or adult were not derived for benzene because of its carcinogenicity. ATSDR has prepared a toxicological profile on benzene (ATSDR, 1991). This draft document did not derive acute, intermediate, or chronic oral MRLs. An acute inhalation MRL of 0.002 ppm was derived for benzene; no intermediate or chronic inhalation MRLs were derived (ATSDR, 1991).

To identify research reports pertinent to the derivation of a provisional chronic RfD for benzene, EPA and ATSDR documents (as cited above) were reviewed; in addition, a computer search of the literature was conducted from the HSDB, RTECS, TSCATS, and TOXLINE (July 1990 to April 1993, oral strategy) databases. The inhalation database was also considered.

REVIEW OF PERTINENT LITERATURE

Data regarding the toxicity of ingested benzene in humans were limited to reports on single exposures (ATSDR, 1991). Several studies reported very serious effects, including death, but did not report dose levels. One study reported very serious neurological effects and death in humans from a single oral dose of approximately 125 mg/kg (Theines and Haley, 1972; as cited in ATSDR, 1991).

Chronic oral studies. Chronic oral studies have been conducted in F344 rats and B6C3F1 mice (NTP, 1986; Huff et al., 1989), and Sprague-Dawley and Wistar rats and Swiss and RF/J mice (Maltoni et al., 1983, 1985, 1989).

In the NTP (1986) study, F344 rats and B6C3F1 mice of both sexes were treated by gavage with benzene, 5 days/week for 103 weeks. Results of this study have also been

reported by Huff et al. (1989). For rats, males (60/group) were administered doses of 0, 50, 100, or 200 mg/kg (0, 36, 71, or 143 mg/kg/day) and females (60/group) were administered doses of 0, 25, 50, or 100 mg/kg (0, 18, 36, or 71 mg/kg/day). Survival decreased with increasing dose in rats of both sexes, and was significantly decreased ($p < 0.05$) at 200 mg/kg in males and at 50 and 100 mg/kg in females. Body weight depression of $\geq 10\%$ relative to controls was observed in male rats treated with 200 mg/kg/day and female rats treated with 100 mg/kg. Dose-related leucopenia was significant ($p < 0.05$) in female rats treated with 25 mg/kg or higher for 3, 6, 9, and 12 months; leukocyte levels were comparable to controls after 15, 18, 21, and 24 months of treatment. In male rats, dose-related leucopenia was significant ($p < 0.05$) at 50 mg/kg or higher for 3, 6, 9, 12, 15, and 18 months. A similar pattern of significant ($p < 0.05$), dose-related decrease, followed by eventual return to control levels, was observed for lymphocyte levels in female rats treated with 25 mg/kg or higher and in male rats treated with 50 mg/kg or higher. Lymphoid depletion was observed in the thymus of 0/44, 4/42, 8/41, and 10/34 male rats treated with 0, 50, 100, and 200 mg/kg benzene, respectively. In the spleen, lymphoid depletion was observed in 0/49, 19/58, 8/47, and 23/47 male rats treated with 0, 50, 100, and 200 mg/kg benzene, respectively, and in 0/50, 11/50, 8/49, and 10/49 female rats treated with 0, 25, 50, and 100 mg/kg benzene, respectively. Increased ($p < 0.05$) incidences of malignant tumors were observed at dose levels of 50 mg/kg or greater in male rats (Zymbal gland carcinomas, squamous cell papillomas and squamous cell carcinomas of the oral cavity, and squamous cell papillomas and squamous cell carcinomas of the skin) and at 25 mg/kg or greater in female rats (Zymbal gland carcinomas, squamous cell papillomas and squamous cell carcinomas of the oral cavity). This study identified a LOAEL of 25 mg/kg (18 mg/kg/day) for leukopenia and lymphocytopenia in female F344 rats treated by gavage for 103 weeks. A LOAEL of 50 mg/kg (36 mg/kg/day) was identified for leukopenia and lymphocytopenia in male F344 rats treated by gavage for 103 weeks. The observed LOAELs were at the lowest dose level tested. Thus, no NOAELs for hematological effects in rats were identified in this study.

In the NTP (1986) study, mice (60/sex/group) were treated by gavage with doses of 0, 25, 50, or 100 mg/kg benzene (0, 18, 36, or 71 mg/kg/day). Survival decreased with increasing dose in mice of both sexes and was significantly decreased ($p < 0.05$) at 100 mg/kg. Body weight depression of $\geq 10\%$ relative to controls was observed in mice of both sexes treated with 100 mg/kg. Significantly ($p < 0.05$) decreased leukocyte counts were observed in males after 3, 6, 9, 12, 15, 18, and 21 months of treatment with 50 and/or 100 mg/kg, but males treated with 25 mg/kg had significantly decreased leukocyte counts only after 6 and 21 months of treatment. In female mice, leucopenia was observed only at 12 and 18 months, in both cases significant ($p < 0.05$) at all treatment levels. Significantly ($p < 0.05$) decreased lymphocyte counts were observed in males after 3, 6, 9, 12, 15, 18, and 21 months of treatment with 50 and/or 100 mg/kg, but males treated with 25 mg/kg had significantly ($p < 0.05$) decreased lymphocyte counts only after 12 months of treatment. In female mice, significant ($p < 0.05$) lymphocytopenia was observed at 25 mg/kg or higher at 12 and 18 months, and at 100 mg/kg at 3 months. Hematopoietic hyperplasia of the bone marrow was observed in 0/49, 11/48, 10/50, and 25/49 male mice treated with 0, 25, 50, or 100 mg/kg, respectively, and in 3/49, 14/45, 8/50, and 13/49 female mice treated with 0,

25, 50, or 100 mg/kg, respectively. Increased splenic hematopoiesis was observed in 5/49, 9/48, 19/49, and 24/47 male mice treated with 0, 25, 50, or 100 mg/kg, respectively, and in 9/49, 10/45, 6/50, and 14/49 female mice treated with 0, 25, 50, or 100 mg/kg, respectively. In the female mice, increased incidences of epithelial hyperplasia of the ovary occurred at all three doses and of senile atrophy of the ovary occurred at the lower two doses compared with controls. Increased ($p < 0.05$) incidences of malignant tumors were observed at 25 mg/kg or higher in both sexes of mice (Zymbal gland squamous cell carcinomas, malignant lymphomas, alveolar/bronchiolar carcinomas, alveolar/bronchiolar carcinomas and adenomas (combined), Harderian gland adenomas, and squamous cell carcinomas of the preputial gland in males and Zymbal gland squamous cell carcinomas, malignant lymphomas, ovarian granulosa cell tumors, ovarian benign mixed tumors, carcinomas and carcinosarcomas of the mammary gland, alveolar/bronchiolar carcinomas, and alveolar/bronchiolar adenomas in females). This study identified a LOAEL of 25 mg/kg (18 mg/kg/day) for leukopenia and lymphopenia in male and female B6C3F1 mice treated by gavage for 103 weeks. The observed LOAELs were at the lowest dose level tested. Thus, no NOAELs for hematological effects in mice were identified in this study.

Beginning in 1976, a series of carcinogenicity studies on oral treatment of rodents with benzene were performed at the Bologna Institute of Oncology, including 52-104 week studies on Sprague-Dawley and Wistar rats and Swiss and RF/J mice. The results of the studies from this laboratory were reported in numerous publications, including Maltoni et al. (1983, 1985, 1989). Limited information regarding non-carcinogenic effects were reported in the various publications since the major emphasis of the studies was the carcinogenic effects of benzene. No statistical information was included in the various publications, making interpretation of the data difficult.

Maltoni et al. (1985) treated Sprague-Dawley rats (13 weeks of age, 30-35/sex/group) by gavage with 0, 50, or 250 mg/kg benzene in oil, 4-5 days/week for 52 weeks, then observed until death; the expanded doses, assuming that the rats were treated an average of 4.5 times/week, were 0, 32, and 161 mg/kg/day, respectively. In addition, Sprague-Dawley rats (7 weeks of age, 40-50/sex/group) were treated by gavage with 0 or 500 mg/kg benzene in oil, 4-5 days/week for 104 weeks, then observed until death; the expanded doses, assuming that the rats were treated an average of 4.5 times/week, were 0 and 321 mg/kg/day, respectively (Maltoni et al., 1985). Maltoni et al. (1983) reported some preliminary information on these studies, including some non-carcinogenic endpoints. Mortality was higher in benzene treated groups and appeared to be dose-related; body weights were not affected. Maltoni et al. (1983) stated that mortality in the first portion of the study was due to direct (toxic) effects of treatment and in the later portion, was partially due to tumors. Mortality was similar to that of controls during treatment with 500 mg/kg for 92 weeks (Maltoni et al., 1983); body weight appeared to be somewhat depressed relative to controls. No further information regarding survival or body weight was provided in the later reports on these studies (Maltoni et al., 1985, 1989). In Sprague-Dawley rats exposed to 500 mg/kg for 84 or 92 weeks, decreased total RBC (only at 92 weeks), WBC, and lymphocytes were observed (Maltoni et al., 1983, 1985). Multiple-site carcinomas developed at 50, 250, and/or 500 mg/kg in rats in these studies. Zymbal gland, oral cavity, nasal

cavity, and skin carcinomas, forestomach tumors, subcutaneous angiosarcoma, mammary gland tumors, hepatomas, non-myeloid leukemias, and other tumors were observed, with greater incidence and more types of malignancies observed at the higher treatment levels.

Additional gavage studies of benzene (at 500 mg/kg, 4-5 days/week) by Maltoni et al. (1989) in Wistar rats, Swiss mice, and RF/J mice focussed entirely on carcinogenic effects, which were similar to those reported in the above studies and occurred in all three strains/species. The report of these studies did not discuss non-carcinogenic effects.

Subchronic oral studies. Subchronic oral studies have been conducted in F344 rats and B6C3F1 mice of both sexes (NTP 1986; Huff et al., 1989), female Wistar rats (Wolf et al., 1956), Charles River CD-1 male mice (Hsieh et al., 1988), and B6C3F1 female mice (White et al., 1984).

NTP (1986) treated F344 rats and B6C3F1 mice (10/species/group/sex; 6-8 weeks of age) with 0, 25, 50, 100, 200, 400, or 600 mg/kg benzene, by gavage in corn oil, 5 days/week for 17 weeks; the expanded doses were 0, 18, 36, 71, 143, 286, or 429 mg/kg/day. An additional 5 animals/species/group/sex were tested at the 0, 200 and 600 mg/kg dose levels and killed at 60 days of treatment. Hematological analyses were performed on all the animals killed at 60 days and on 5 animals/species/group/sex at the end of the study. Comprehensive histopathologic examinations were performed on all the animals killed at 60 days and on animals in the control and 600 mg/kg groups at the end of the study. In addition, necropsies were performed on all animals and the spleens of all animals were examined histopathologically. Results of this study have also been reported by Huff et al. (1989).

No compound-related deaths were observed for rats. Final body weight depression of $\geq 10\%$ relative to controls was observed in male and female rats at dose levels of 200 mg/kg and greater. Significant ($p < 0.05$) leukopenia and lymphocytopenia were observed in male and female rats after 60 days of treatment with 200 or 600 mg/kg (the only treatment groups tested on day 60). On day 120 of treatment, significant ($p < 0.05$) leucopenia and lymphocytopenia were observed in female rats at 25 mg/kg and higher and significant ($p < 0.05$) lymphocytopenia was observed in male rats at 400 mg/kg (blood counts were performed on only 1 male given 600 mg/kg for 120 days). Lymphoid depletion of B-cells in the spleen was observed in 100% of male and female rats exposed to 600 mg/kg for 60 or 120 days, and in 3/5 male and 4/5 female rats exposed to 200 mg/kg for 60 days. Increased extramedullary hematopoiesis in the spleen was observed in 4/5 male and 3/5 female rats treated with 600 mg/kg for 120 days. Incidences of lymphoid depletion of B cells and extramedullary hematopoiesis in the spleen were not reported for controls (or other groups); the implication was that these conditions were seen only in the groups for which incidences were given. This study identified a LOAEL of 25 mg/kg (18 mg/kg/day) in female rats and LOAEL of 200 mg/kg (143 mg/kg/day) in male rats for hematological effects following treatment by gavage for 17 weeks. The observed LOAEL for female rats was at the lowest

dose level tested. Thus, the study does not define a NOAEL for hematological effects in rats.

NTP (1986) reported no compound-related deaths in the mice; final body weight depression of $\approx 7\%$ was seen at ≥ 100 mg/kg. Tremors were observed intermittently in male and female mice treated with 400 or 600 mg/kg. No leukopenia or lymphocytopenia was observed in male or female mice after 60 days of treatment with 200 or 600 mg/kg. At 120 days, significant ($p < 0.05$) leukopenia and lymphocytopenia were observed in male mice at dose levels of 50 mg/kg and greater, and in female mice at 400 (only lymphocytopenia) and 600 mg/kg. A NOAEL of 25 mg/kg (18 mg/kg/day) and a LOAEL of 50 mg/kg (36 mg/kg/day) for hematological effects were identified in male mice treated by gavage for 17 weeks. A NOAEL of 200 mg/kg (143 mg/kg/day) and a LOAEL of 400 mg/kg (286 mg/kg/day) for hematological effects were identified in female mice treated by gavage for 17 weeks.

White et al. (1984) exposed female B6C3F1 mice (12/group; 6-7 weeks of age) to benzene in drinking water (containing emulphor to increase solubility of benzene) at exposure levels of 0, 50, 1000, and 2000 mg/L (0, 12, 195, or 350 mg/kg/day, respectively) for 30 days. Body weight was significantly ($p < 0.05$) decreased, relative to controls, at the high-exposure level. A dose-related ($p < 0.01$) decrease in absolute and relative spleen weight was observed, significant at the high-exposure level ($p < 0.01$). In one test, spleen cellularity was reported to be significantly ($p < 0.05$) decreased at all exposure levels, and in a separate test, at only the mid- and high-exposure levels. Dose-related ($p < 0.05$) leukopenia and lymphocytopenia were observed, significant ($p < 0.05$) at the mid- and high-exposure level. A dose-related ($p < 0.01$) decrease in eosinophils was observed, significant ($p < 0.05$) at the high-exposure level. At the high-exposure level, significant ($p < 0.05$) decreases in levels of erythrocytes and hemoglobin, and significant ($p < 0.05$) increases in mean corpuscular volume and mean corpuscular hemoglobin were observed. No exposure-related effects were observed for levels of blood urea nitrogen, serum creatinine, serum glutamic oxaloacetic transaminase, or serum glutamic pyruvic transaminase, indicators of renal and hepatic damage. Dose-related ($p < 0.05$) changes were observed in immunological tests on spleen cells and in assays of bone marrow: decreases were observed with respect to IgM antibody forming cells/spleen in response to sheep RBC, lymphocyte proliferation response to the T cell mitogen Con A and the B cell mitogen LPS, number of T lymphocytes, and femoral CFU-GM; an increase was observed in bone marrow cell DNA synthesis. These effects were not significant at 12 mg/kg/day, but were dose-related ($p < 0.05$) and significant ($p < 0.05$) at 195 and/or 350 mg/kg/day. Of all the immunological indices tested, only one endpoint (stimulation index for lymphocyte proliferation of spleen cells in response to medium containing 0.5 $\mu\text{g/ml}$ of Con A) was significantly ($p < 0.05$) decreased at 12 mg/kg/day. The number of B lymphocytes was not affected, but the investigators commented that the number of B lymphocytes in the controls was lower than for historical controls for their laboratory. This study identifies a marginal NOAEL of 12 mg/kg/day and a LOAEL of 195 mg/kg/day for hematological and immunological effects in mice exposed to benzene in drinking water for 30 days; the 12 mg/kg/day exposure level may approach the threshold of toxicity, as significance ($p < 0.05$) was seen for two effects at this exposure

level, and numerous hematological and immunological effects that were dose-related, but not significant at the 12 mg/kg level, were observed.

Hsieh et al. (1988) treated male Charles River CD-1 mice (5/group; 6-7 weeks of age) with benzene in the drinking water at exposure levels of 0, 40, 200, or 1000 mg/L (0, 8, 40, or 180 mg/kg/day, respectively) for 28 days. The treatment had no adverse effects with respect to mortality, clinical signs, body weight change, liver weight, or gross necropsy. A dose-related decrease in relative spleen weight was observed, significant ($p < 0.05$) at the high-exposure level. In one test, spleen cellularity was reported to be significantly ($p < 0.05$) decreased at all exposure levels, and in a separate test, only at the high-exposure level. Although relative thymus weights were decreased at all exposure levels, the values were not statistically significantly different from controls. Dose-related hematological effects (erythrocytopenia, leucopenia, lymphocytopenia, increased mean corpuscular volumes) were observed at all exposure levels, significant at $p < 0.05$; hematocrit was significantly ($p < 0.05$) decreased at the mid- and high-exposure levels. The authors indicated that the increased mean corpuscular volume, and decreased hematocrit and numbers of RBC were indicative of severe macrocytic anemia. Biphasic responses were observed in immunological tests [mitogen-stimulated (LPS, PWM, Con A, PHA) splenic lymphocyte proliferation, mixed splenic lymphocyte culture response to allogenic YAC-1 cells, cytotoxic splenic T-lymphocyte response to allogenic YAC-1 cells], with a significantly ($p < 0.05$) increased response at the low-exposure level, and significantly ($p < 0.05$) decreased responses at the mid- and/or high-exposure level. Using several methods to determine primary antibody response to sheep RBC, significantly ($p < 0.05$) decreased responsiveness was observed at the mid- and/or high-exposure levels; this response was either significantly ($p < 0.05$) increased or not different from controls in mice exposed to the low-exposure level. This study identifies a LOAEL of 8 mg/kg/day (the lowest treatment level tested) for hematological and immunological effects in male mice exposed to benzene in drinking water for 30 days. No NOAEL for hematological effects in mice were identified in this study.

Wolf et al. (1956) treated female Wistar rats (10/group) by gavage with benzene in olive oil, 5 days/week for 6 months. The reported doses were 0, 1, 10, 50, or 100 mg/kg/day, but it was not clear whether these represented the dose on treatment days or the dose expanded from 5 to 7 days/week. The usual practice in the primary literature is to report the actual gavage doses given on treatment days. Assuming that the usual practice was followed for this study, the expanded doses would be 0, 0.7, 7.1, 35.7, and 71.4 mg/kg/day, respectively. Parameters measured included mortality, clinical signs, body and organ weights, hematology, blood biochemistry, bone marrow counts, and gross and microscopic pathology of lungs, heart, liver, kidneys, spleen, testes, adrenals and pancreas. Leucopenia (described as "very slight") was reported for 10 mg/kg; at higher dose levels erythrocytopenia and leucopenia were observed. No quantitative data or statistical analysis were reported. The authors reported that rats fed 1 mg/kg had "no evidence of ill effects" with respect to gross appearance, growth, periodic blood counts, blood urea nitrogen, average final body and organ weights, histopathological examination, and bone marrow counts. For higher treatment levels, only adverse effects were described, requiring the assumption that no adverse effects were observed with respect to the other tested parameters.

This study identified a NOAEL of 1 mg/kg (0.7 mg/kg/day) and a LOAEL of 10 mg/kg (7.1 mg/kg/day) for hematological effects in female rats treated by gavage for 6 months.

Developmental and reproductive toxicity studies. Developmental toxicity studies of orally administered benzene have been conducted in rats (Exxon Chemical Company, 1986) and mice (Nawrot and Staples, 1980; Seidenberg et al., 1986, as cited in ATSDR, 1991).

Exxon Chemical Company (1986) treated bred female Sprague-Dawley rats (20-22/group) by gavage with 0, 50, 250, 500, or 1000 mg/kg/day on gestation days 6-15. No dose-related mortality was observed. Significant ($p \leq 0.05$) findings in the treated dams as compared with controls were decreased food consumption at 250, 500 and 1000 mg/kg, decreased body weights and body weight gains at 500 or 1000 mg/kg/day, and increased incidence of alopecia at 1000 mg/kg. Developmental toxicity was limited to decreased ($p \leq 0.05$) fetal body weights in the 500 and 1000 mg/kg/day groups. Fetuses were examined only for external malformations, not for skeletal and visceral malformations. This study identified a NOAEL of 50 mg/kg/day and LOAEL of 250 mg/kg/day for maternal toxicity and tentative NOAEL of 250 mg/kg/day and LOAEL of 500 mg/kg/day for developmental toxicity in Sprague-Dawley rats.

Nawrot and Staples (1980) treated bred CD-1 mice (23-105/group) by gavage with benzene in cottonseed oil at dose levels of 0, 0.3, 0.5, or 1 ml/kg/dose, 3 times daily, on gestation days 6-15. Using a specific gravity of 0.8765 g/ml (ATSDR, 1991) and multiplying by 3 doses/day results in doses of 0, 789, 1315, and 2630 mg/kg/day, respectively. Additional groups of mice were similarly treated with 0 or 1 ml/kg/dose (0 or 2630 mg/kg/day) on gestation days 12-15. Mortality rates in dams treated with 0, 789, 1315, and 2630 mg/kg/day were 2/105, 0/27, 6/48, and 7/23, respectively. Significant ($p < 0.05$) findings in the dams included the increased mortality at the mid- and high doses, increased liver weights at the mid- and high doses, increased relative liver weights at all three doses, and a reduction in maternal weight gain only at the low dose. A dose-related decrease in apparent pregnancy rate at sacrifice was observed, significantly different ($p < 0.05$) from controls at all dose levels; at the mid and high doses, this effect resulted from early resorption of entire litters ($p < 0.05$). The decrease in apparent pregnancy rate at the low dose was attributed to an unusually high pregnancy rate in vehicle controls. Fetal body weights were decreased in all dose groups treated on days 6-15. In dams exposed on gestation days 12-15, no deaths occurred; significant ($p < 0.05$) results included increased absolute maternal liver weight, decreased maternal weight gain and fetal body weight and increased number of resorptions. No increases relative to controls in external, visceral or skeletal defects were seen in any of the treatment groups. This study identified a LOAEL of 789 mg/kg/day for developmental toxicity and possible maternal toxicity resulting from treatment on gestation days 6-15, and a LOAEL of 2630 mg/kg/day for maternal and developmental toxicity resulting from treatment on gestation days 12-15. The LOAELs were at the lowest (or only) treatment level used and no NOAELs were identified in this study.

In a developmental toxicity screening study, Seidenberg et al. (1986, as cited in ATSDR, 1991) treated bred mice by gavage with benzene in oil at dose levels of 0 or 1300 mg/kg/day on gestation days 8-12. Fetal body weights were decreased; no other effects were reported. This study identifies a LOAEL of 1300 mg/kg/day for developmental toxicity in mice.

Additional developmental toxicity studies of benzene have been conducted in animals using inhalation exposure. These studies identify hematopoietic effects in the animal fetus (Keller and Snyder, 1986, 1988) as a sensitive developmental toxicity endpoint for inhalation exposure to benzene.

Reproductive studies of orally administered benzene were not located in the literature searched. The NTP (1986) study reported increased incidences of hyperplasia and senile atrophy of the ovary in female B6C3F1 mice at ≥ 25 mg/kg (18 mg/kg/day) in the chronic oral study of benzene.

Histopathological changes in the testes in rabbits (Wolf et al., 1956) and testicular lesions and ovarian cysts in mice (Ward et al., 1985) were reported following exposure to relatively high concentrations of benzene by inhalation. A study on reproductive effects in female rats was conducted by the inhalation route. In this study, female Sprague-Dawley rats (26/group) were exposed to vapor concentrations of 0, 1, 10, 30, or 300 ppm benzene (0, 3, 32, 96, or 958 mg/m³), 6 hours/day, 5 days/week during pre-mating (10 weeks) and mating periods, then 6 hours/day, 7 days/week, on gestation days 1-20, and lactation days 5-21 (Bio/dynamics, 1980). The following parameters were used to assess toxicity: clinical signs, mortality rate, body weight gain, pregnancy rates, and gestation length in dams; number alive and dead at birth, sex distribution, survival, body weights, organ weights, and gross necropsy in pups. The treatment had no adverse effects with respect to reproduction or maternal toxicity. No additional information on reproductive effects is available from the inhalation database.

DERIVATION OF PROVISIONAL CHRONIC RfD

The critical effects of orally administered benzene were determined to be hematotoxicity and immunotoxicity, probably related to the adverse effects of benzene on hematopoiesis (Wolf et al., 1956; White et al., 1984; NTP, 1986; Hsieh et al., 1988; Huff et al., 1989). This was not unexpected, because the extensive database on inhalation toxicity of benzene identifies hematological, hematopoietic and immunological toxicity as the critical effect, supported by human and animal data. The Health Risk Technical Support Center has derived a provisional RfC based on a free-standing NOEL of 0.045 ppm for hematological effects in occupationally exposed humans (Collins et al., 1991). Although no LOAEL was identified in this study, other occupational exposure studies reported hematological and/or hematopoietic effects at higher concentrations (Aksoy et al., 1971; Fishbeck et al., 1978), as did inhalation studies in experimental animals. Immunological effects have been reported in short-term inhalation studies of benzene in animals.

The NTP (1986) chronic and subchronic toxicity studies on rats and mice were not used as the basis for the RfD because the lowest dose tested, 18 mg/kg/day, was a LOAEL for hematological effects, and is higher than LOAELs for similar effects observed in other studies of subchronic duration. Wolf et al. (1956) reported very slight leukopenia in rats treated by gavage for 6 months with 10 mg/kg (7.1 mg/kg/day) and leukopenia and erythrocytopenia at higher dose levels; no effects were observed at 1 mg/kg (0.7 mg/kg/day). Hsieh et al. (1988) reported hematological and immunological effects in male CD-1 mice exposed to 8 mg/kg/day benzene in the drinking water. A marginal NOAEL of 12 mg/kg/day was identified for hematological and immunological effects in female B6C3F1 mice exposed to benzene in the drinking water (White et al., 1984); the 12 mg/kg/day exposure level may approach the threshold of toxicity, as discussed previously.

The 28-day study by Hsieh et al. (1988) was chosen as the principal study because it demonstrated significant ($p < 0.05$) hematological and immunological toxicity. The lowest dose (8 mg/kg/day) was identified as a minimal LOAEL, because this dose enhanced the immune parameters measured in the study. The two higher doses significantly depressed immune function. This study examined primarily hematological and immunological effects; no effects were seen on clinical chemistry indices of renal and hepatic toxicity. The 6-month study by Wolf et al. (1956) was chosen as a co-principal study because it provides supporting information for the critical effect and threshold for toxicity. The LOAEL for hematological effects was 7.1 mg/kg/day, with a NOAEL of 0.7 mg/kg/day. No adverse effects were observed for non-hematological endpoints, including blood biochemistry, bone marrow counts, and gross and microscopic pathology of major tissues and organs. The Wolf et al. (1956) study was not chosen as the principal study because the results were presented only as a summary; actual data and statistical analysis were not reported. Results from chronic (NTP, 1986) and other subchronic studies (White et al., 1984; NTP, 1986) support the critical effects (hematological, immunological) identified in the principal and co-principal studies.

A provisional RfD of 3E-4 mg/kg/day was determined based on the NOAEL of 0.7 mg/kg/day from the study by Wolf (1956). An uncertainty factor of 3000 was applied to account for interspecies (10) and intraspecies (10) differences, extrapolation from a subchronic study (10), and insufficient database (3). Uncertainty regarding deficiencies in the database is small because of the reasonably adequate oral database, which includes developmental toxicity studies, and the extensive supporting inhalation database, but some uncertainty remains due to the lack of a two-generation reproductive study.

Confidence in the principal study is medium to low. The critical effect (hematological and immunological) was investigated through the use of a battery of tests and a range of dose levels, appropriate statistical analyses were performed, a dose-effect relationship was established, and the LOAEL is consistent with the LOAEL and NOAEL from a 6 month study (Wolf et al., 1956) measuring hematological effects and other endpoints of toxicity. Confidence in the principal study is limited by the small group sizes (5 animals/dose), testing of only one sex, short duration, and limited range of endpoints examined. Confidence in the database is medium because the critical effect is supported by numerous studies on benzene by the oral and inhalation routes and for various durations, including chronic; confidence in the database is not higher because of the lack of a two-generation reproductive study. Reflecting the medium to low confidence in the key study and medium confidence in the database, confidence in this provisional RfD is medium.

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**Risk Assessment Issue Paper for:
Derivation of a Provisional Chronic Inhalation RfC
for Benzene (CASRN 71-43-2)**

INTRODUCTION

To identify research reports pertinent to the derivation of a provisional chronic RfC for benzene, EPA and ATSDR documents on benzene (U.S. EPA, 1980; 1984; 1989; 1993a,b; 1994a,b; ATSDR, 1991) and the HSDB, RTECS, and TSCATS databases were reviewed; in addition, a computer search of the literature was conducted (TOXLINE, June, 1986 - February, 1992).

OSHA lists an PEL TWA of 1 ppm; however, some segments of industry are exempt from the 1 ppm standard, and instead have a PEL TWA of 10 ppm (OSHA, 1989). ACGIH lists a TLV TWA of 10 ppm; however a TLV TWA of 0.1 ppm has been proposed and is awaiting verification (ACGIH, 1991). The NIOSH REL (10-hour TWA) is 0.1 ppm (NIOSH, 1991).

TOXICITY IN HUMANS

There is an extensive database on the toxicity of inhaled benzene. Secondary sources, as well as literature searches, were used to identify studies that defined the thresholds of toxicity. Specifically, we looked for studies that evaluated subchronic, chronic, developmental, and reproductive toxicity of inhaled benzene. When numerous studies were available, we chose those for which toxic effects were observed at low concentrations of benzene. A number of epidemiology studies were available regarding chronic toxicity of inhaled benzene; four are reported herein, covering a wide range of exposure levels and effects.

Aksoy et al. (1971) examined hematological parameters in 217 apparently healthy male workers (mean age 24.7 years) exposed to 30-218 ppm benzene (96-696 mg/m³) for 3 months to 17 years, and in 100 male hospital workers and medical students (mean age 26.6 years). Peripheral blood samples were obtained for measurement of RBC, WBC, PCV, platelets, and differential counts. In 11 benzene-exposed workers known to have hematological abnormalities, bone marrow samples were obtained for determination of cellularity and myeloid and erythroid series. Twenty-four percent of the exposed workers had hematological abnormalities, including leukopenia (9.7%), thrombocytopenia (1.84%), leukopenia associated with thrombocytopenia (4.6%), pancytopenia (2.76%), acquired pseudo-Pelger-Huet anomaly (0.46%), lymphocytosis (0.46%), giant platelets (0.46%),

eosinophilia (2.3%), basophilia (0.46%), and eosinophilia associated with basophilia (0.46%). Low hemoglobin levels, PCV, and MCV, indicative of mild or moderate hypochromic or normochromic anemia, were observed in 33% of the benzene-exposed workers. In bone marrow tests, 9/11 workers had hematopoietic abnormalities, including hypercellularity (in 1 worker), hypocellularity (4), and maturation arrest (8) and vacuolization (4) in the erythroid and myeloid series. This study identifies a LOAEL of 30 ppm (96 mg/m³) for hematopoietic effects in humans.

Kipen et al. (1989) examined about 18,000 peripheral blood counts from hematologic surveillance records on 459 workers employed in the rubber industry between 1940 and 1975. Mean concentrations of benzene decreased from 137 to 66 ppm between 1940 and 1948, with a mean 8-hour TWA of 75 ppm (239.6 mg/m³). WBC counts increased from 1940-1948 and were positively correlated with the decreasing benzene levels. Between 1948-1975 workers were exposed to mean 8-hour TWA concentrations of 15-20 ppm (48-64 mg/m³). WBC counts in blood samples from workers exposed from 1948-1975 were not correlated with changing benzene exposure levels. These data suggest that benzene exposure in the 75 ppm (239.6 mg/m³) range influences WBC count in exposed workers, whereas exposure to benzene at 15-20 ppm (48-64 mg/m³) does not influence WBC count.

Collins et al. (1991) examined hematological parameters (peripheral blood RBC, WBC, hemoglobin, platelets, and MCV) in workers (n=200) exposed to benzene over a 10-year period. Within this 10-year period the mean length of exposure was 7.3 years. The workers were exposed to an 8-hour TWA of 0.01-1.40 ppm benzene. The mean TWA exposure was 0.045 ppm (J. Collins, 1992, personal communication). A group (n=268) of non-benzene exposed workers in the same plant were used as controls. There were statistically significant differences on demographic (age, race, sex) and personal habit (currently smokers, regular exercise) variables between the benzene-exposed workers and the control group. Multiple regression analyses were applied using the confounding factors and current exposure as independent variables. No significant correlations between cumulative exposure and hematological parameters were identified. Thus, this study identifies a free-standing NOEL of 0.045 ppm (0.14 mg/m³) for hematological effects in humans.

Fishbeck et al. (1978) examined hematological parameters (RBC, WBC, hematocrit, hemoglobin, mean corpuscular volume, platelets, differential blood counts, clot retention determinations, sedimentation rate, and blood indices) in 10 employees exposed to high benzene concentrations [8-hour TWA of >25 ppm (>80 mg/m³)] for 2.5-22.9 years, with an average of 9.6 years of exposure. Concentrations of benzene in the work area were especially high in 1963, with the 8-hour TWA ranging from 37-132 ppm (118-422 mg/m³); after 1963, conditions were altered to assure that concentrations of benzene remained below 25 ppm (80 mg/m³) (the acceptable limit at that time). Examination of the 10 employees in 1963 revealed enlarged RBC's, high MCV (10/10), slightly low hemoglobin levels (9/10), and transient anemia; bone marrow was examined at this time and no abnormalities were found. After 1963, hematological values for these employees improved (in 1977, 5/10 workers had increased MCV values) and by 1978 none of the employees had developed serious health problems. The authors concluded that exposure of workers to high levels of

benzene produced transient hematological effects, which did not influence the long-term overall health of the workers.

TOXICITY IN ANIMALS

Male C57BL/J mice (sample size not reported; initial age 8 weeks) were exposed via inhalation to vapor concentrations of 0 or 10 ppm benzene (0 or 32 mg/m³) for 6 hours/day, 5 days/week for up to 178 days (Baarson et al., 1984). After 32, 66, and 178 days of exposure, peripheral blood samples were obtained from all mice for determination of levels of RBC, lymphocytes, and neutrophils, and 5 mice/exposure level were sacrificed for measurement of erythroid progenitor cells [colony forming units - erythroid (CFU-E), burst forming units (BFU-E), nucleated red cells, and total cellularity] in bone marrow and spleen. There was a significant ($p < 0.05$) decrease in levels of RBC (at 66 and 178 days) and lymphocytes (at all sampling times) in peripheral blood of benzene-exposed mice. CFU-E and BFU-E in bone marrow were significantly ($p < 0.01$) decreased at all sacrifice times and at 66 days, respectively; after 178 days of treatment, bone marrow CFU-E was 5% of controls. Splenic CFU-E (10% of controls), nucleated red cells (15%), and total nucleated cellularity (84%) were significantly ($p < 0.05$) decreased in mice sacrificed at 178 days. This study identifies a LOAEL of 10 ppm (32 mg/m³) for depressed hematopoiesis in mice.

Male CD-1 mice (11-12/exposure level/exposure duration; 8-12 weeks of age) were exposed for 6 hours/day, 5 days/week to vapor concentrations of 0 or 9.6 ppm (0 or 31 mg/m³, respectively) benzene for 10 weeks or to 0 or 302 ppm (0 or 966 mg/m³) benzene for 26 weeks (Green et al., 1981a,b). On the day of the last exposure, samples (pooled from groups of 3-4 mice) were obtained from the peripheral blood, bone marrow and the spleen to evaluate hematological and hematopoietic cells. In mice exposed to 9.6 ppm (31 mg/m³), no adverse effects were observed with respect to mortality, body weights, or cells in the peripheral blood or bone marrow. Splenic weight, total nucleated cells per spleen, and nucleated red blood cells per spleen were significantly ($p < 0.05$) increased in mice exposed to 9.6 ppm (31 mg/m³). Mice exposed to 302 ppm (966 mg/m³) had the following significant ($p < 0.05$) changes: increased mortality rate; decreased numbers of lymphocytes and RBC in peripheral blood; decreased numbers of lymphocytes, granulocytes, multipotential hematopoietic stem cells, and committed granulocyte/macrophage progenitor cells in bone marrow; decreased splenic weight, and numbers of lymphocytes, multipotential hematopoietic stem cells and committed granulocyte/macrophage progenitor cells in the spleen; increased incidence of atypical cell morphology in peripheral blood, bone marrow, and spleen. This study identifies a NOAEL of 9.6 ppm (31 mg/m³) for slight hematopoietic effects in mice exposed to benzene for 10 weeks and a LOAEL of 302 ppm (966 mg/m³) for severe hematopoietic toxicity in mice exposed for 26 weeks.

Sprague-Dawley rats (50/sex/group; 12 weeks of age) and CD-1 mice (150/sex/group; 9 weeks of age) were exposed to nominal vapor concentrations of 0, 1, 10, 30, or 300 ppm benzene (99.9% purity) (0, 3, 32, 96, or 959 mg/m³), 6 hours/day, 5 days/week, for 13 weeks (Ward et al., 1985). Clinical observations and body weight data were normal in both

species. High-exposure level rats had leukopenia and significantly ($p < 0.05$) decreased femoral marrow cellularity. High-exposure level mice had leukopenia, anemia, thrombocytopenia, and significant increases in MCV, MCH, glycerol lysis time, and incidence and severity of morphological changes in RBC. Relative testes weights were significantly decreased in high-dose male mice. High-dose mice had histological abnormalities in the thymus (atrophy), bone marrow (myeloid hyperplasia), lymph nodes (lymphoid depletion of mesenteric and mandibular lymph nodes; plasma cell infiltration into mandibular lymph node), spleen (increased incidence of extramedullary hematopoiesis; periarteriolar lymphoid sheath depletion), ovaries (bilateral ovarian cysts), and testes (bilateral atrophy/degeneration; decreases in spermatozoa in the epididymal ducts; increased numbers of abnormal sperm types); similar lesions were observed in the testes and ovaries of mice exposed to concentrations lower than 300 ppm (959 mg/m³), but the authors did not consider these effects to be biologically significant. The incidence and severity of most benzene effects were greater in male mice than in female mice. This study identifies a NOAEL of 30 ppm (96 mg/m³) and a LOAEL of 300 ppm (959 mg/m³) for these effects in rats and mice.

Male C57BL/6 mice were exposed to 0 or 300 ppm benzene (0 or 960 mg/m³), 6 hours/day, 5 days/week for 9 weeks (Baarson and Snyder, 1991). Blood was withdrawn from the tail vein for differential white blood cell counts and peripheral nucleated red cell counts. Following sacrifice, femurs and the spleen were aseptically removed and placed in sterile culture dishes. Single cell suspensions were made, and the numbers of colony forming unit erythroid (CFU-E) cells and burst forming unit erythroid (BFU-E) cells were counted in control vs. exposed animals. From 1 day after beginning of exposure to benzene until the end of the treatment period, peripheral red blood cell counts were decreased. In addition, the numbers of BFU-E and CFU-E colonies were depressed to less than half the control values, in all exposed animals at days 5 and 60 of exposure. These effects represent an adverse effect on the organism, due to the potential for anemia. In this study, the combined treatment of benzene and ethanol was studied in a second group of animals, and was the thrust of the new information from this group. The exposure levels are far greater in this study than in previous work; a LOAEL of 960 mg/m³ (HEC=171 mg/m³) was established for hematotoxic effects.

Similar results were seen in a study where female C57BL/6xDBA/2 F1 hybrid mice were exposed to 0 or 300 ppm benzene (0 or 960 mg/m³), 6 hours/day, 5 days/week for 6-7 weeks (Vacha et al., 1990). Indices of hematopoiesis were measured in peripheral blood (RBC and WBC count, Hb, Hct, reticulocyte, and leukocyte count), in addition to ⁵⁹Fe accumulation in the erythropoietic organs (spleen and bone marrow) and in the peripheral RBC's. The distribution of developmental classes of erythroblasts was also determined. This study found that animals became anemic after 6-7 weeks of benzene exposure. The number of erythroblasts in the bone marrow was not different, however exposure to benzene shifted the population to a less mature class of cells. The number of colonies derived from BFU-E and CFU-E were decreased to 70% and 34% of controls, respectively. A LOAEL of 960 mg/m³ (HEC=171 mg/m³) was established for hematotoxic effects.

BDF1 mice were exposed to 0, 100, 300, or 900 ppm benzene (0, 320, 960, 2880 mg/m³) for up to 4 weeks (Seidel et al., 1990). The numbers of hematopoietic progenitor cells, early and late progenitors (BFU-E, CFU-E), and granuloid progenitors (CFU-C) were determined. A group was generated to establish the effect of ethanol (drinking water) on these effects. This study demonstrated that the number of CFU-E per femur was decreased in a concentration-dependent manner by benzene. This effect was evident at 300 and 900 ppm (960 and 2880 mg/m³, respectively) concentrations, however the effect of the 100 ppm (320 mg/m³) exposure group was uncertain, as the study focused on the effect of ethanol on benzene toxicity. The LOAEL/NOAEL was thus difficult to determine.

Male Sprague-Dawley rats (40/group) were exposed to vapor concentrations of 0 or 100 ppm benzene (0 or 319 mg/m³), 6 hours/day/ 5 days/week, for life (American Petroleum Institute, 1983). Blood samples were obtained at 2-4 week intervals throughout the treatment period. The treatment had no adverse effects with respect to mortality rates or body weight gain. Peripheral erythrocyte and lymphocyte counts were depressed at nearly every sampling time in treated rats, but the extent of decrease was not statistically significant at $p < 0.05$. Significantly increased incidence of splenic hyperplasia ($p < 0.005$) and hemosiderin pigments ($p < 0.001$) were observed in benzene-exposed rats. The incidences of normally rare tumors in treated rats were liver (4/40), Zymbal gland (2/40), and chronic myelogenous leukemia (1/40); the authors considered these tumors to be related to the benzene exposure. This study identifies a LOAEL of 100 ppm (319 mg/m³) for slight hematological effects in rats.

Male AKR/J (50/group) and C57BL/6J mice (40/group) were exposed to vapor concentrations of 0, 100 (319 mg/m³; AKR mice only), or 300 ppm (958 mg/m³; C57BL/J mice only) benzene, 6 hours/day, 5 days/week for life (Snyder et al., 1980). The following parameters were used to assess toxicity: clinical signs (observed daily), body weights (measured biweekly), hematology (RBC, WBC, WBC differentials, absolute neutrophil and lymphocyte; measured biweekly in 10 control and 10 treated mice from each strain), and gross and microscopic necropsy (lung, liver, spleen, kidney, and bone marrow). The treatment had no adverse effects with respect to life span, body weight, or incidence of lymphoma in AKR mice. Treated AKR mice had significant (± 2 standard errors) degrees of lymphocytopenia, neutrophilia, erythropenia, and bone marrow hypoplasia ($p < 0.05$). Treated C57BL mice had significant (± 2 standard errors) degrees of lymphocytopenia, neutrophilia, erythropenia, morphological changes in peripheral blood cells, and bone marrow and splenic hyperplasia ($p < 0.05$). The incidence of hematopoietic neoplasms was significantly ($p < 0.05$) increased in C57BL mice, including 6 cases of thymic lymphoma. This study identifies a LOAEL of 100 ppm (319 mg/m³) for hematopoietic effects in mice.

Pregnant Swiss Webster mice (5/exposure level/progeny age group; initial age 8-12 weeks) were exposed via inhalation to nominal vapor concentrations of 0, 5, 10, or 20 ppm benzene (0, 16, 32, or 64 mg/m³) for 6 hours/day on gestation days 6-15 (Keller and Snyder, 1988). On gestation day 16 (fetuses), 2 days after birth (neonates), and 6 weeks after birth (adults), progeny (1-2 males and 1-2 females/litter) were sacrificed to determine the amounts and types of hemoglobin produced, and hemopoietic cells in the peripheral blood and hematopoietic organs. No evidence of maternal or non-hematopoietic developmental toxicity

was observed in treated mice, and no adverse hematopoietic effects were observed in fetuses. The treatment had no adverse effects in any progeny with respect to peripheral blood levels of RBC, MCH, blasts, dividing granulocytes, lymphocytes, or ratio of hemoglobin A major to hemoglobin A minor. There was a concentration-related decrease in peripheral blood levels of early nucleated red cells in neonates, significant ($p < 0.05$) at all exposure levels. High-exposure level neonates had significantly ($p < 0.05$) increased numbers of nondividing granulocytes and decreased numbers of late nucleated red cells in peripheral blood. In high-exposure level neonates, hepatic levels of blasts, dividing granulocytes, non-dividing granulocytes, and lymphocytes were significantly ($p < 0.05$) increased and late nucleated red cells were significantly ($p < 0.05$) decreased; hepatic levels of blasts were also significantly ($p < 0.05$) increased at the low-exposure level in neonates. In adults, there was a concentration-related decrease in early nucleated red cells in bone marrow, significant ($p < 0.05$) at the high-exposure level. High-exposure level adults also had significant ($p < 0.05$) increases in splenic levels of blasts, dividing granulocytes, and nondividing granulocytes; splenic levels of non-dividing granulocytes were also increased in low-exposure level adults. This study identifies a LOAEL of 5 ppm (16 mg/m³) for developmental hematopoietic effects in mice.

Pregnant Swiss-Webster mice (5/exposure level/progeny age group; initial age 8-12 weeks) were exposed via inhalation to nominal vapor concentrations of 0, 5, 10, or 20 ppm benzene (0, 16, 32, or 64 mg/m³) for 6 hours/day on gestation days 6-15 (Keller and Snyder, 1986). On gestation day 16 (fetuses), 2 days after birth (neonates), and 6 weeks after birth (adults), progeny (1-2 males and 1-2 females/litter) were sacrificed for measurement of hematopoietic progenitor cells [colony forming units - erythroid (CFU-E), burst forming units - erythroid (BFU-E), and granulocytic colony forming cells (GC-CFU-C)] from the liver (fetuses and neonates), and bone marrow and spleen (adults). In addition, 10-week old progeny from litters in the control and mid-exposure group were exposed for 2 weeks to 10 ppm (32 mg/m³) benzene, then sacrificed for measurement of hematopoietic progenitor cells from the bone marrow and spleen. There was no evidence of maternal or non-hematopoietic developmental toxicity in benzene-exposed mice. There was a significant ($p < 0.05$) increase in the numbers of erythroid burst forming units from livers of male and female fetuses exposed to the low- and mid-exposure level, respectively. The following significant ($p < 0.05$) changes were observed with respect to CFU-E: in fetuses, there were increases in liver CFU-E at the low- and mid-exposure levels and decreases at the high-exposure level; in male neonates, there were increases and decreases in liver CFU-E at the mid-exposure level, and increases at the high-exposure level; in adult mice there were decreases in bone marrow CFU-E and increases in spleen CFU-E in males exposed to 10 ppm (32 mg/m³) in utero. Liver GM-CFU-C in neonates was significantly ($p < 0.05$) decreased at the mid-exposure level (males only) and increased at the high-exposure level. Mice exposed to 10 ppm (32 mg/m³) benzene in utero and for 2 weeks as adults had significantly ($p < 0.05$) decreased bone marrow CFU-E (males only) and splenic GM-CFU-C; mice exposed to air in utero and 10 ppm (32 mg/m³) benzene for 2 weeks as adults had no changes in bone marrow or splenic CFU-E, but had a significant ($p < 0.05$) decrease in splenic GM-CFU-C (females only). The authors concluded that benzene treatment in utero induced hematopoietic alterations in

fetuses, persisting until at least 10 weeks after birth. This study identifies a LOAEL of 5 ppm (16 mg/m³) for developmental hematopoietic effects in mice.

Bred Sprague-Dawley rats (17-20/group; initial body weights 210-223 g) were exposed via inhalation to nominal vapor concentrations of 0, 10, 50, or 500 ppm benzene (0, 32, 160, and 1600 mg/m³) for 7 hours/day, on gestation days 6-15, followed by sacrifice on gestation day 20 for determination of developmental abnormalities (Kuna and Kapp, 1981). The treatment had no adverse effects on dams with respect to mortality rate, hematology, or gross necropsy. Body weight gain over gestation days 5-15 was significantly ($p < 0.05$) decreased in mid- and high-exposure level dams. Fetal body weight was significantly ($p < 0.05$) decreased at the mid- and high-exposure levels and fetal crown-rump length was decreased at the high-exposure level. The number of litters with skeletal and visceral variants was significantly ($p < 0.05$) increased at the mid- and high-exposure levels. The skeletal and visceral abnormalities observed included exencephaly, angulated ribs, dilated lateral and third ventricles of the brain, forefeet ossification out of sequence, generalized lagging ossification, and decreased numbers of caudals, and metacarpals, metatarsals, and phalanges/foot; the authors considered these abnormalities to be related to the benzene treatment. This study identifies a NOAEL of 10 ppm (32 mg/m³) and a LOAEL of 50 ppm (160 mg/m³) for maternal toxicity and developmental effects in rats.

Bred Sprague-Dawley rats (26-31/group) were exposed to nominal vapor concentrations of 0, 10, or 40 ppm benzene (0, 32, or 128 mg/m³) for 6 hours/day on gestation days 6-15 (Litton Bionetics, 1978). The treatment had no adverse effects on mortality rate, body weight gain, or food consumption in dams. Pregnancy ratio, fetal weight, live litter size, and incidence of variants and malformations were similar in control and treatment groups. Benzene-exposed rats had significantly ($p < 0.05$) decreased ratio of live fetuses/implantation site. The number of resorption sites was increased in benzene-exposed rats, but the difference was only significant ($p < 0.05$) in the low-exposure group. This study identifies a LOAEL of 10 ppm (32 mg/m³) for developmental effects in rats.

Female Sprague-Dawley rats (26/group) were exposed to vapor concentrations of 0, 1, 10, 30, or 300 ppm benzene (0, 3, 32, 96, or 958 mg/m³), 6 hours/day, 5 days/week during pre-mating (10 weeks) and mating periods, then 6 hours/day, 7 days/week, on gestation days 1-20, and lactation days 5-21 (Bio/dynamics, 1980). The following parameters were used to assess toxicity: clinical signs, mortality rate, body weight gain, pregnancy rates, and gestation length in dams; number alive and dead at birth, sex distribution, survival, body weights, organ weights, and gross necropsy in pups. The treatment had no adverse effects with respect to reproduction or maternal toxicity. This study identifies a NOAEL of 300 ppm (958 mg/m³) for reproductive effects and maternal and developmental toxicity in rats.

Coate et al. (1984) exposed pregnant Sprague-Dawley rats (40/group) to 1, 10, 40, or 100 ppm benzene (3.2, 32, 128, or 320 mg/m³), 6 hr/day, days 6-15 of gestation. No maternal toxicity or teratogenic effects were noted. There was no deviation from controls in the number of resorptions. There was reduced fetal weight at 100 ppm ($p < 0.05$) thus, a

LOAEL of 100 ppm (320 mg/m³) can be identified along with a NOAEL of 40 ppm (128 mg/m³).

Unovary and Tatrai (1985) exposed pregnant CFLP mice (15/group) and New Zealand rabbits (11-15/group) to 0, 160 or 320 ppm benzene (0, 500 or 1000 mg/m³), 24 hr/day, during days 6-15 (mice) or 7-20 (rabbits) of gestation. There were no teratogenic effects in either species. In rabbits, exposure to 320 ppm (1000 mg/m³) was associated with reduced fetal weight ($p < 0.05$) in the presence of reduced maternal body weight gain. The NOAEL and LOAEL for this effect was 160 ppm (500 mg/m³) and 320 ppm (1000 mg/m³), respectively. Mice exposed to concentrations ≥ 160 ppm (500 mg/m³) had reduced fetal weight, as well as skeletal deformities (maternal weight data not provided). The LOAEL for this effect was 160 ppm (500 mg/m³).

DERIVATION OF PROVISIONAL CHRONIC INHALATION RfC

Chronic exposure of humans to benzene vapor in the work place resulted in hematological and/or hematopoietic effects at concentrations of 30-218 ppm (96-697 mg/m³) (Askoy et al., 1971; Fishbeck et al., 1978; Kipen et al., 1989). At lower concentrations (0.01-20 ppm; 0.03-64 mg/m³), no adverse hematological effects were observed in peripheral blood of humans (Kipen et al., 1989; Collins et al., 1991). However, it is not known whether chronic exposure to low concentrations of benzene affects hematopoiesis in the bone marrow and spleen in humans.

The most sensitive endpoint for long-term exposure to benzene vapor is toxicity to hematopoietic progenitor cells. The lowest LOAEL identified for this effect are at 10 ppm (32 mg/m³) in mice exposed to benzene subchronically (Baarson et al., 1984). Green et al. (1981a,b) identified NOAELs for damage to hematopoietic progenitor cells at 10 ppm (32 mg/m³). Ward et al. (1985) established a NOAEL of 30 ppm (96 mg/m³) for hematological effects, but effects on the progenitor cells were not evaluated. Lifetime studies provide evidence that mice are more sensitive to the long-term effects of benzene than are rats (Snyder et al., 1980; American Petroleum Institute, 1983). Reproductive effects (testicular lesions and ovarian cysts) were observed in mice exposed to 300 ppm (959 mg/m³) for 13 weeks, but not in mice exposed to 30 ppm (96 mg/m³) (Ward et al., 1985), or in female rats exposed to 300 ppm (959 mg/m³) for 17 weeks during pre-mating, mating, gestation, and lactation (Bio/dynamics, 1980). The Keller and Snyder (1988) developmental toxicity study identified a LOAEL of 5 ppm (16 mg/m³), however the LOAEL_{HEC} of 16 mg/m³ is higher than the LOAEL_{HEC} of 5.7 mg/m³ from the Baarson et al. (1984) and Green et al. (1981a,b) subchronic studies.

The Baarson et al. (1984) study was selected as the critical study because the exposure period was longer (25 vs. 10 weeks) at the low dose of 10 ppm (32 mg/m³) than that of the Green et al. (1981a,b) studies.

- a. LOAEL of 10 ppm (32 mg/m³) from the Baarson et al. (1984) studies was adjusted for intermittent exposure:

$$\text{LOAEL}_{\text{ADI}} = 32 \text{ mg/m}^3 \times 6 \text{ hours/24 hours} \times 5 \text{ days/7 days} = 5.7 \text{ mg/m}^3.$$

- b. Derivation of the LOAEL_{HEC}:

$$\text{LOAEL}_{\text{HEC}} = \text{LOAEL}_{\text{ADI}} \times L_A/L_H$$

where: L_A = blood:air partition coefficient for benzene in male B6C3F1 mice (12.1) (Gargas et al., 1989)

L_H = blood:air partition coefficient for benzene in humans (8.19) (Gargas et al., 1989)

$\text{LOAEL}_{\text{HEC}} = 5.7 \text{ mg/m}^3 \times 1.0 = 5.7 \text{ mg/m}^3$; because the ratio of animal to human blood:air partition coefficients is greater than 1 (1.48), the default ratio of 1 is used (U.S. EPA, 1987).

An uncertainty factor of 1000 was applied to the LOAEL_{HEC} of 5.7 mg/m³ to yield a provisional RfC of $6 \times 10^{-3} \text{ mg/m}^3$. The uncertainty factor includes 3 for interspecies extrapolation using dosimetric adjustments, 10 for intraspecies variability, 10 for use of a subchronic study, and 3 to extrapolate from a minimal LOAEL.

Confidence in the key study (Baarson et al., 1984) is low. A small number of animals of one sex were used. Green et al. (1981a,b) identified NOAELs at the same dose level for similar endpoints. Confidence in the database is medium. A large number of studies corroborated the hematopoietic effects observed in the Baarson et al. (1984) study. In addition, testicular lesions were reported by Ward et al. (1985); however, male reproductive performance tests and/or a multigeneration reproduction study were not identified. Reflecting the low confidence in the key study and medium confidence in the database, confidence in this provisional chronic RfC is low.

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Benzene Chronic RfC Principal/Supporting Studies - Inhalation Exposure

Study	Species/ Route	Conc. (ppm/ mg/m ³)	Duration	Critical Effect	NOAEL (mg/m ³)	LOAEL (mg/m ³)	NOAEL[adj] LOAEL[adj] (mg/m ³)	HEC (mg/m ³)
Co-critical Studies:								
Baarson et al. 1984 (chronic)	C57BL/J mice	0/0 10/32	6 h/d 5 d/wk 178 d	Decr. ability of mouse marrow progenitor cells to form colonies	None	32	5.7 L	5.7 L
Green et al. (1981a,b) (acute and chronic)	CD-1 mice	0/0 9.8/31	6 h/d 5 d/wk 10 wk	Hematolo- gical	31	None	5.5 N	5.5 N
		0/0 302/966	26 wk					
Supporting Studies:								
Aksoy et al. 1971	Human	0/0 30/96 218/696	3 m to 17 yr	Hematolo- gical	None	96	34 L	
Collins et al. 1991	Human	0/0 0.045/0.14	7 yr	None	0.14 (free- standing)	None	0.05 N	

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Study	Species/ Route	Conc. (ppm/ mg/m ³)	Duration	Critical Effect	NOAEL (mg/m ³)	LOAEL (mg/m ³)	NOAEL[adj] LOAEL[adj] (mg/m ³)	HEC (mg/m ³)
Ward et al. 1985 (subchronic)	CD-1 mice	0/0 1/3 10/32 30/96 300/959	6 h/d 5 d/wk 13 wk	Hematological effects (decr. RBC, WBC, platelets, Hb, M/E ratios, and Hct.; Histological effects (91 d postexp): testicular atrophy, abn. sperm, decr. spermatozoa, etc.	96	959	17 N 171 L	17 N 171 L
	Rats			Decr. lymphocyte count, incr. neutrophils; Histological effects: decr. femoral marrow cellularity (at 7 d exposure)	96	959	17 N 171 L	17 N 171 L
Baerson and Snyder 1991 (subchronic)	C57BL/6 male mice	0/0 300/960	6 h/d 5 d/wk 9 wk	Decreased RBC count; decr. ability of mouse marrow	progenitor cells to form colonies	None	960	171L

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Study	Species/ Route	Conc. (ppm/ mg/m ³)	Duration	Critical Effect	NOAEL (mg/m ³)	LOAEL (mg/m ³)	NOAEL[adj] LOAEL[adj] (mg/m ³)	HEC (mg/m ³)
Vacha et al. 1990 (subchronic)	C57BL/6x DBA/2 F1 hybrid female mice	0/0 300/960	6 h/d 5 d/wk 6-7 wk	Decreased RBC count; decr. ability of mouse marrow progenitor cells to form colonies	None	960	171 L	171 L
Seidel et al. 1990 (subchronic)	BDF1 mice	0/0 100/320 300/960 900/2880	6 h/d 5 d/wk 4 wk	Decreased ability of mouse marrow progenitor cells to form colonies	Unclear	960	171 L	171 L
Kuna and Kapp 1981 (develop- mental)	Sprague- Dawley rats	0/0 10/32 50/160 500/1600	7 hr/d GD 6-15	Decr. fetal body weight	32	160	32 N 160 L	32 N 160 L
Coate et al. 1984 (develop- mental)	Sprague- Dawley rats	1/3.2 10/32 40/128 100/320	6 hr/d GD 6-15	Decr. fetal body weight	128	320	128 N 320 L	128 L 320 L

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Study	Species/ Route	Conc. (ppm/ mg/m ³)	Duration	Critical Effect	NOAEL (mg/m ³)	LOAEL (mg/m ³)	NOAEL[adj] LOAEL[adj] (mg/m ³)	HEC (mg/m ³)
Unovary and Tatrai 1985 (develop- mental)	CFLP mice	0/0 160/500 320/1000	24 hr/d GD 6-15	Decr. fetal body weight	None	500	500 L	500 L
	New Zealand white rabbits		GD 7-20	Decr. fetal body weight	500	1000	500 N 1000 L	500 N 1000 L

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August 31, 1995

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ASSISTANCE REQUESTED: Systemic and Carcinogenic Toxicity Information for Multiple Chemicals (*Masterlist / Region IV*)

ENCLOSED INFORMATION: Attachment 1: Risk Assessment Issue Paper for: Derivation of a Provisional Subchronic Inhalation RfC for Chloroform (CASRN 67-66-3)

Attachment 2: Risk Assessment Issue Paper for: Derivation of a Provisional Subchronic Inhalation RfC for Chloromethane (CASRN 74-87-3)

Attachment 3: Risk Assessment Issue Paper for: Evaluation of the Carcinogenicity of 1,2,4-Trimethylbenzene (CASRN 95-63-6)

Attachment 4: Risk Assessment Issue Paper for: Derivation of a Provisional RfC for Trimethylbenzene (1,2,4 and 1,3,5) (CASRN 108-67-8 and 95-63-6)

BE ADVISED: It is to be noted that the values provided in the attached Risk Assessment Issue Papers are **provisional only**, and have not been through the U.S. EPA's formal review process. Therefore, they do not represent a U.S. EPA verified assessment. If you have any questions regarding

this information, please contact Joan Dollarhide at (513)
569-7539.

Attachments

cc:

**Risk Assessment Issue Paper for:
Derivation of a Provisional Subchronic Inhalation RfC for Chloroform (CASRN 67-66-3)**

The presented data in Torkelson et al. (1976) are a compilation of several smaller studies that were conducted prior to 1965. Groups of rats (n=10–12/sex/dose), guinea pigs (n=8–12/sex/dose), and rabbits (n=2–3/sex/dose) were exposed to air or chloroform at target concentrations of 25, 50, or 85 ppm for 7 hours/day, 5 days/week for 195–203 days. The average actual concentrations were 23.1, 47.5, and 84.4 ppm (113, 232, and 412 mg/m³). The duration-adjusted concentrations were 23.4, 48.3, and 85.8 mg/m³. One male and one female dog were exposed to an actual concentration of 23.1 ppm (duration-adjusted value 23.4 mg/m³) for the same exposure period. Male rats (10/group) were also exposed to an actual concentration of 23.1 ppm for 1, 2, or 4 hours/day, 5 days/week, for the same period (duration-adjusted concentrations 3.36, 6.72, and 13.4 mg/m³). Strains were not reported for any species. Actual concentrations were generally within 10% of target. All animals were sacrificed the day after the last exposure, except that half of the rats exposed to 23.1 ppm 7 hours/day were kept for 6 weeks before being sacrificed. Unexposed control groups were also used.

Survival of many of the control groups was very low. Survival of unexposed male rats in one set of experiments was 67%, and survival of air-exposed male guinea pigs in one set of experiments was 50%. Too few rabbits were tested to derive meaningful survival figures, but in some rabbit control groups only 1/3 or 2/3 animals survived. No mention was made in the report of the high control mortality rates, and no mention was made of any possible cause (e.g. bacterial or viral infections). The high mortality in the controls compromises the study and makes it difficult to interpret the survival data from the treatment groups. For example, although only 40% of the rats exposed to 23.1 ppm for 4 hours/day survived, the effect was not concentration-related. In addition, no adverse effects on organ weight or histomorphology were reported for this group or the rats exposed to 23.1 ppm for 1 or 2 hours/day. An infection in the animal colonies could account for both the high mortality and the inconsistent results in guinea pigs and rabbits discussed below.

Excess mortality (40%, compared with a high of 17% for the concurrent controls) was reported in male rats exposed to 84.4 ppm. The mortality was reported to be generally attributable to pneumonia. Marked interstitial pneumonitis was seen in males at this level. Pneumonitis was also reported in female guinea pigs and male rabbits at this level ($_{HEC}$ for guinea pigs based on thoracic surface area = 77 mg/m³) and in female rabbits at 23.1 ppm ($_{HEC}$ = 15 mg/m³), but not at higher concentrations.

Relative liver weight was significantly elevated in male rats exposed to 84.4 ppm, but not in either gender of any other species. Marked central lobular granular degeneration of the liver was

reported in rats of both sexes that were exposed to 84.4 ppm. Similar degeneration was noted at 47.5 ppm, and some degeneration, along with focal areas of necrosis, occurred in male rats only at 23.1 ppm. Liver enzymes (serum glutamic-pyruvic transaminase (SGPT) and alkaline phosphatase (AP)) were normal for all exposure groups. No histopathology was reported in rats that were allowed to recover for 6 weeks prior to sacrifice. Inconsistent results were obtained with guinea pigs and rabbits. Central lobular granular degeneration with foamy vacuolization was observed in male guinea pigs exposed to the low concentration (23.1 ppm), and female guinea pigs at this exposure level had light-colored livers with central foamy vacuolization. No liver effects were seen in guinea pigs at higher exposure levels. In rabbits, liver effects were observed in high exposure males (foamy vacuolization and necrosis) and females (central lobular granular degeneration and necrosis), and low exposure females (central lobular granular degeneration and necrosis with slight fibrosis in the portal area). No effects were seen in rabbits of either sex exposed to 47.5 ppm, or in male rabbits exposed to the low concentration.

Kidney weights of male rats in the two higher-exposure groups were significantly increased; increases in female rats at these levels were not statistically significant. Cloudy swelling of the renal tubular epithelium occurred in rats of both sexes and at all exposure concentrations. There was no effect on serum urea nitrogen (SUN). Male guinea pigs in the low-concentration group had increased tubular and interstitial nephritis; the absolute and relative kidney weights of female guinea pigs exposed at this level were elevated without accompanying histopathology. No kidney effects were seen at higher levels. Similarly, interstitial and tubular nephritis was reported in male and female rabbits at the low concentration, but no kidney effects were seen in rabbits at 47.5 ppm, and the only kidney effects reported at the high concentration was cloudy swelling in females. Female rabbits at 23.1 ppm also exhibited glomerular nephritis. The only pathological effect in the dogs was an increase in capsular space in the glomeruli and marked cloudy swelling of the renal tubular epithelium observed in the female.

This study suggests a LOEL_{HEC} of 23.4 mg/m³ and a NOAEL_{HEC} of 13.4 mg/m³ for rats. Inconsistent results preclude the designation of a NOAEL or LOAEL for the other species in this study.

Plummer et al. (1990) exposed male black-hooded Wistar rats (n=12/group) to air or 50 ppm (244 mg/m³) chloroform continuously for 4 weeks, except for two 1½ hour periods/week. An additional group was exposed discontinuously (6 h/d, 5 d/wk) to 275 ppm (1343 mg/m³), for the same total exposure (duration-adjusted concentration of 240 mg/m³). Within each group, rats received either plain drinking water, 0.05% w/v sodium phenobarbitone, or 5% v/v 1,3-butanediol (n=4/subgroup). Only the liver was examined histologically. The most prominent effect was microvesicular fatty change, seen through-out zone 2, often in zone 1, and usually not in zone 3. There was also some focal necrosis throughout the acini; quantitation revealed a significant difference from the control (p<0.01). Adverse effects were smaller in the discontinuous-exposure group. Injury in this group was characterized as minor to mild, with scattered hepatocytes containing small fat droplets and a few foci of liver cell necrosis. The smaller adverse effect of discontinuous exposure was attributed to saturation of the metabolic processes that convert chloroform to toxic intermediates.

In this study 240 mg/m³ is the LOAEL, but only one concentration was tested.

Murray et al. (1979) exposed 34–40 bred CF-1 mice to filtered air or 100 ppm spectral grade chloroform (488 mg/m³) for 7 hr/day on gestation days (gd) 1–7, 6–15, or 8–15. Maternal body weight gain was significantly reduced in the groups exposed on gd 1–7 and gd 8–15, and slightly reduced in the group exposed from gd 6 to gd 15; food consumption was slightly reduced in all treatment groups. One exposed mouse in the latter group died of gastric ulceration, but the etiology of this effect was unknown. The ability to maintain pregnancy (percentage of animals that had implantation sites at sacrifice) was significantly reduced ($p < 0.05$) in the groups exposed on gestation days 1–7 or 6–15. The number of resorptions/litter was significantly increased in the group exposed on gd 1–7, but not in the other exposed groups. Mean fetal body weight and crown-to-rump length was significantly reduced ($p < 0.05$) in the groups exposed on gestation days 1–7 or 8–15, but not in the group exposed on gestation days 6–15. The incidence of cleft palate was significantly increased ($p < 0.05$) only in the group exposed on gd 8–15. The effect occurred largely in fetuses with retarded growth. All exposed groups exhibited an increased incidence of delayed ossification of the skull. Liver toxicity was observed in the dams. Absolute and relative liver weights were significantly increased ($p < 0.05$) in the groups exposed on gestation days 6–15 or 8–15, and SGPT activity was significantly increased in the only group in which it was measured (those exposed on gestation days 6–15).

Schwetz et al. (1974) also found that chloroform increased the incidence of resorptions and structural defects. They exposed bred Sprague-Dawley (Spartan) rats ($n = 20-31$ for treated groups; $n = 77$ for the control) for 7 hr/day on gestation days 6–15 to filtered air or 30, 95, or 291 ppm reagent grade chloroform (0, 146, 464, or 1421 mg/m³). An additional control group was fed only 3.7 g food/day to control for the marked anorexia observed in the high-exposure group. In the high-exposure group, the apparent conception rate was only 15% (compared to 88% in the control), and the percent resorptions was significantly increased ($p < 0.05$). Since the only evidence of implantation was a focal increase in the vascularity of the mesometrium, chloroform exerted its toxic effect early in gestation. Both the fetal body weight and fetal crown-to-rump length were significantly reduced ($p < 0.05$) in the offspring of the high-exposure group. Gross abnormalities (acaudia or short tail and imperforate anus) were significantly ($p < 0.05$) elevated in the 95 ppm exposure group. Additional significant effects in the 95 ppm group were increases ($p < 0.05$) in the incidence of delayed sternebra ossification, wavy ribs and soft tissue edema. The only effects observed in the "starved" control group were reduced fetal size, showing that the effects at 291 ppm were largely chemical-related, rather than secondary to weight loss. Relative maternal liver weight was significantly increased at 95 and 291 ppm. This study suggests a NOAEL for developmental effects of 146 mg/m³.

In an oral study, Munson et al. (1982) administered 0, 50, 125, or 250 mg/kg-day chloroform by gavage to CD-1 mice ($n = 7-12$ /sex/dose). A dose-dependent increase in relative liver weight that was statistically significant only at the high dose was observed in males. In females, absolute and relative liver weight was significantly increased at all three doses. A dose-dependent increase in SGPT and serum glutamic-oxalacetic transaminase (SGOT) levels was observed in males, but the effect was not statistically significant. Slight histopathology was observed in the kidneys and livers

of males and females. Generalized hydropic degeneration of hepatocytes and occasional small focal collections of lymphocytes were observed in the liver. The kidney showed small intertubular collections of chronic inflammatory cells. The report did not state at which doses histopathology was observed. Humoral immunity was affected, as by decreased antibody-forming cells in the spleen. The effect was statistically significant only in males at the high dose. Cell-mediated immunity (delayed-type hypersensitivity) was impaired in females at the high dose. No histopathology was observed in the spleen. Based on the liver effects, the LOAEL is 50 mg/kg-day.

In an experiment conducted by Heywood et al. (1979), beagle dogs (8/sex/dose) received 15 or 30 mg/kg-day in a toothpaste vehicle by gelatin capsule, vehicle alone, or were untreated. Statistically significant increases in SGPT levels were found at 30 mg/kg throughout the treatment period. A smaller but statistically significant increase was found at 15 mg/kg from weeks 130 through 364. Levels declined in the posttreatment recovery period. Apparent dose-related increases were also observed in gamma glutamyl transferase (GGT) and glutamic dehydrogenase (GDH), additional markers of liver injury. Relative liver weight was only slightly increased in both treatment groups. The only treatment-related histopathological changes was an increase in the size and number of fatty liver cysts in both treatment groups and animals of both sexes. The incidence of haemosiderin occurrence was also increased in the treated animals. This study suggests a LOAEL of 15 mg/kg-day.

Palmer et al. (1979) treated Sprague-Dawley rats (10/sex/dose) with chloroform in toothpaste or toothpaste only by oral gavage. No primary data were presented. Increased liver weight with fatty change and necrosis were reported at 410 mg/kg/day, along with gonadal atrophy and bone marrow proliferation. There were "less pronounced" changes, along with changes in liver and kidney weight at 150 mg/kg-day. Study details are insufficient to establish a NOAEL or LOAEL. In a followup study, Sprague-Dawley rats (50/sex/dose) received 60 mg/kg-day or vehicle only by gavage for 95 weeks. Animals of both sexes exhibited a small but consistent and progressive reduction in weight gain. The study authors did not report if the change was statistically significant. Plasma cholinesterase was significantly lowered in treated females at weeks 29, 34, and 52 ($p < 0.001$) and at week 80 ($p < 0.01$). The decrease was not significant at week 95. Relative liver weight was significantly reduced in treated females, but only "minor" histological changes were noted. Because only one dose was tested, no NOAEL can be determined.

Thompson et al. (1974) observed mild fetotoxicity at oral doses that were maternally toxic in rats. They administered 20, 50, or 126 mg/kg-day chloroform (twice-daily) in corn oil or vehicle alone to bred Sprague-Dawley rats (25/dose) on gestation days 6-15. Maternal toxicity (alopecia, rough appearance, and weight loss) was observed in the high-dose group. Reduced body weight gain was also seen at 50 mg/kg-day. The only evidence of fetal toxicity was reduced fetal weight at 126 mg/kg-day ($p < 0.05$). The incidence of bilateral extra lumbar ribs was significantly increased at the high dose. Dutch-Belted rabbits (15/dose) were given daily gavage doses of chloroform in corn oil or vehicle alone. There was no treatment-related maternal toxicity. Fetal toxicity, as evidenced by reduced fetal weight ($p < 0.05$) was observed at 20 and 50 mg/kg-day. Fetal weight at 35 mg/kg-day was also reduced, but the effect was not statistically significant. There were no treatment-related

major anomalies. Incomplete ossification of skull bones was significantly elevated in the two lower dosing groups.

DERIVATION OF A PROVISIONAL SUBCHRONIC RfC

A NOAEL of 113 mg/m³ (23.1 ppm) was determined based on focal areas of liver necrosis and cloudy swelling of the renal tubular epithelium from subchronic rat inhalation studies by Torkelson et al. (1976). Calculation of the human equivalent concentration (HEC) for the NOAEL is as follows:

- a. NOAEL of 113 mg/m³ is adjusted for intermediate exposure:

$$\begin{aligned}\text{NOAEL}_{\text{ADJ}} &= 113 \text{ mg/m}^3 \times 4 \text{ hours}/24 \text{ hours} \times 5 \text{ days}/7 \text{ days} \\ &= 13.4 \text{ mg/m}^3\end{aligned}$$

- b. Derivation of the NOAEL_{HEC}:

$$\begin{aligned}\text{NOAEL}_{\text{HEC}} &= \text{NOAEL}_{\text{ADJ}} \times (\text{b:a } \lambda(\text{a})/\lambda(\text{h})) \\ &= 13.4 \text{ mg/m}^3\end{aligned}$$

where: NOAEL_{HEC} was calculated for a gas:extrarrespiratory effect, assuming periodicity was attained.

b:a $\lambda(\text{a}) = 20.8$, b:a $\lambda(\text{h}) = 20.8$, (Gargas et al., 1989).
Since b:a $\lambda(\text{a})$ is greater than b:a $\lambda(\text{h})$, a default value of 1.0 was used for this ratio.

Calculation of the provisional subchronic RfC follows:

$$\begin{aligned}\text{Subchronic RfC} &= \text{NOAEL}_{\text{HEC}} / \text{UF} \times \text{MF} \\ &= 13.4 \text{ mg/m}^3 / 300 \times 1 \\ &= 4\text{E-}2 \text{ mg/m}^3\end{aligned}$$

The uncertainty factor of 300 includes a 10 for protection of sensitive human subjects, 3 for interspecies extrapolation, and 10 for lack of a complete database including reproductive toxicity endpoints not being fully addressed and poor quality of the studies (consistently high mortality even in control groups). The resulting provisional subchronic RfC is 4E-2 mg/m³.

The study by Torkelson et al. (1976) looked at three species (rat, rabbit and guinea pig) at several dose levels and found liver and kidney effects in all three species using established histopathological and hematological methods. However, high mortality in some control groups and some treatment groups, as well as inconsistent results with rabbits and guinea pigs, reduce the

confidence in this study. The finding of liver effects is supported by the results of Plummer et al. (1990), who compared the effects on the liver of continuous and discontinuous exposure to equivalent concentrations of chloroform. Liver and kidney histopathology were also found in rats following oral dosing with chloroform (Munson et al., 1982). Increased liver weight and liver enzymes levels, and slight liver histo-pathology was found in beagles after 7.5 years of oral dosing (LOAEL = 15 mg/kg-day; Heywood et al., 1979). Species differences in metabolism following oral dosing have been noted (Davidson et al., 1982). Davidson et al. (1982) noted that there are important differences between humans and animals in the pharmacokinetics and metabolism of orally-administered chloroform. It is not known whether similar differences exist in metabolism of inhaled chloroform. The most common effect of acute human exposure to chloroform has been reported to be hepatic damage (U.S. EPA, 1985). Confidence in the database is medium. The resulting confidence in the subchronic RfC is medium.

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(10/26/93)

**Risk Assessment Issue Paper for:
Derivation of a Provisional Subchronic Inhalation RfC
for Chloromethane (CASRN 74-87-3)**

PRINCIPAL STUDY

A 90-day subchronic exposure of Fischer 344 rats and B6C3F1 mice was conducted in a study by the Chemical Industry Institute of Toxicology (CIIT) (1979). Animals (10/sex/group) were exposed to 0, 360, 741, or 1473 ppm chloromethane (0, 743, 1530, or 3042 mg/m³), 6 hours/day, 5 days/week. Significant increases in SGPT activity were observed in male mice in the 1473 ppm group (3041 mg/m³, HEC=543). This was supported by histopathological liver changes (hepatic infarction and cytoplasmic vacuolar alterations of hepatocytes) found on necropsy, and increased liver weight in this concentration group. Cytoplasmic vacuolar changes were found in 7/19 (37%), 7/18 (39%), and 9/14 (64%) of mice in the 0, 748, and 1473 ppm groups, respectively. An increase in the severity of this effect was also evident in the high-concentration group. Therefore, a LOAEL of 1473 ppm (3041 mg/m³, HEC=543) and a NOAEL of 741 ppm (1530 mg/m³, HEC=273) were established for hepatotoxicity on the basis of this study. The brains of exposed mice were not reported to have any compound-related lesions, however no detailed examination of the CNS was conducted.

Body weight of male and female rats in the 1473 ppm (3042 mg/m³, HEC=543) group was significantly lower than control from week 3 to the end of the test period. The magnitude of this effect was approximately 25% at the end of the 90 day exposure period. Body weight gain was not different between control and exposed animals, however. No respiratory or liver effects occurred with chloromethane exposure in rats.

ADDITIONAL STUDIES

Neurotoxicity is one of the hallmarks of chloromethane exposure. Chloromethane-induced neurotoxicity was induced in workers exposed for 2 to 3 weeks (12-16 hours/day) to 265-300 ppm (547-619 mg/m³) in the workplace (Scharnweber et al. 1974). Symptoms of neurotoxicity included blurry vision, headache, disturbances in balance, staggering, deficiency in recent memory, hand tremors, confusion, slurring of speech, numbness, loss of concentration, difficulty in driving, and anorexia. No effects on the respiratory system were reported.

In another short-term study, male and female volunteers were exposed to 100 ppm chloromethane (206 mg/m³), 7.5 hours/day for 5 days. Neurological and behavioral tests revealed no adverse effects (NOEL HEC=46 mg/m³) (Stewart et al. 1977).

Groups of male cats and beagle dogs (3/group) were exposed to 0, 200, or 500 ppm methyl

chloride (0, 413, 1032 mg/m³) for 23.5 hours/day, for 3 days (McKenna 1981b). Neurotoxic effects (ataxia, paralysis, tremor) as well as lesions in the brain stem and spinal cord were observed in dogs exposed to 500 ppm (1032 mg/m³) chloromethane. No effects were observed in cats.

Other evidence for the neurotoxicity found with exposure to chloromethane was shown in the less-than-subchronic exposures of mice in Morgan et al. (1982), and in Landry et al. (1985), discussed below.

Groups of male and female C3H, C57BL/6 and B6C3F1 mice (5/sex/strain/group) were exposed to 500, 1000, 2000, or 3500 ppm chloromethane (1032, 2065, 4130, 7228 mg/m³), 6 hours/day, 5 days/week for 9 exposures. Hepatocellular degeneration occurred in C3H and C57BL/6 mice, with a LOAEL of 500 ppm (1032 mg/m³, HEC=184) for this effect. Cerebellar degeneration occurred at 1000 ppm (2065 mg/m³, FEL HEC=369 mg/m³). Degeneration and necrosis of the renal proximal convoluted tubules were observed at 1000 ppm (2065 mg/m³, HEC=369 mg/m³) in C3H mice and at 2000 ppm (4130 mg/m³, HEC=738 mg/m³) in the other two mouse strains (Morgan et al., 1982).

Groups of 12 female C57BL/6 mice were exposed intermittently (I) or continuously (C) to chloromethane for 11 days. One group was exposed for 5.5 hours/day to 0, 150, 400, 800, 1600, or 2400 ppm (310, 826, 1652, 3304, or 4956 mg/m³) and the second group was exposed for 22 hours/day to 0, 15, 50, 100, 150, or 200 ppm (31, 103, 206, 310, or 413 mg/m³). Mice were subjected to neurofunctional testing (rotating rod training and testing), in addition to gross and histopathological examination at study termination. Mortality was greater in mice exposed to 150 ppm-C. Neurological deficits were detected in 800 ppm-I and 150 ppm-C groups. However, histopathological effects (degenerative changes in the cerebellum) occurred at concentrations of 400 ppm-I and 100 ppm-C. In addition, mice exposed to these concentrations had increased liver toxicity (decreased hepatocyte size due to glycogen depletion) which progressed to focal necrosis after continuous exposure to higher concentrations. The LOAELs for cerebellar degeneration and hepatotoxicity were 400 ppm-I (826 mg/m³, HEC=189 mg/m³) and 100 ppm-C (206 mg/m³; HEC=188 mg/m³). Kidney toxicity was detected in animals exposed to 2400 ppm-I but not after continuous exposure to chloromethane. The NOAELs for these effects were 150 ppm-I (150 mg/m³) or 50 ppm-C (103 mg/m³) (Landry et al., 1985).

McKenna et al. (1981a), exposed Sprague-Dawley rats (10/sex/group) and Beagle dogs (4 males/group) to 0, 50, 150, or 400 ppm chloromethane (0, 105, 314, or 836 mg/m³), 6 hours/day, 5 days/week for approximately 90 days. Rats had a sensory and motor function battery performed. Performance in the wire maneuver test (a test of muscular strength; rats are tested for their ability to raise their hindquarters to the top of a wire while grasping it with their forelimbs) was consistently lower in the 400 ppm exposure group than in controls. During the final 30 days of the study, performance was significantly lower in the 150 ppm exposure group also. Alterations in this parameter were not reflected in concomitant neuromuscular incoordination or other neurologic deficit. There was an increase in relative liver weight in the male rats exposed to 400 ppm chloromethane, which was attributed to the test compound. No histopathologic changes accompanied this effect.

No compound-related changes in mortality, hematology, clinical chemistry, urinalysis, or gross/microscopic pathology were found in this study in exposed dogs. However, dogs had lung lesions that were suggestive of parasitic infection. Liver histopathology revealed slightly swollen hepatocytes in 0/4, 2/4, 1/4, and 2/4 control, 50, 150, and 400 ppm exposure animals. Due to the lack of organ weight changes of biochemical correlate, these changes were considered incidental and were not considered compound-related.

In the chronic mouse and rat study (CIIT, 1981), there was evidence for neurotoxic effects in mice. Impaired rear clutch response and degeneration and atrophy of the cerebellar granular layer were observed in mice at 1000 ppm after 18 months of exposure (2065 mg/m³, HEC=369 mg/m³). The NOAEL for this effect is 225 ppm (465 mg/m³). Kidney tumors (renal cortical adenocarcinomas, papillary cystadenomas, tubular cystadenocarcinomas, tubular cystadenomas, and papillary cystadenocarcinomas) were found in male mice (0/67 controls, 0/61 at 50 ppm, 2/57 at 225 ppm, and 18/82 at 1000 ppm). The testes were the major organ affected in the rat by chloromethane exposure. Atrophy and degeneration of the seminiferous tubules was found in rats exposed to 1000 ppm chloromethane (2065 mg/m³, HEC=369) at the 18 and 24 month sacrifices. A NOAEL of 225 ppm (465 mg/m³) was established for testicular effects in rats. No neuropathological effects were found in any rats.

F-344 rats and C57BL/6 mice were exposed to 0, 100, 500, or 1500 ppm chloromethane (0, 206, 1032, or 3097 mg/m³) for 6 hours/day on gestational days 7-19 (rats) or 6-17 (mice) (Wolkowski-Tyl et al. 1983a). The LOAEL for maternal and fetal toxicity in rats (reduced maternal and fetal body weight gain, decreased fetal crown-rump length) is 1500 ppm (3097 mg/m³). No teratogenicity or alterations in behavior were observed in rats at this concentration.

Mouse dams at 1500 ppm (3097 mg/m³) had neuropathologic signs (difficulty in righting and hunched posture, tremors); animals were sacrificed on gestation days 10-14, and histopathological examination revealed granular cell degradation in the cerebellum, with no other histopathology noted. Fetuses from the 500 ppm (1032 mg/m³) exposure group had an abnormal incidence of cardiac anomalies; there was a reduction or absence of atrioventricular valves, chordae tendineae, and papillary muscles in 6/17 litters. Animals in the 1500 ppm concentration group were not examined for this effect because of their early termination.

C57BL/6 mice were exposed to 0, 250, 500, or 750 ppm chloromethane (0, 516, 1032, 1549 mg/m³), 6 hours/day on gestation days 6-18 (Wolkowski-Tyl et al. 1983b). This study verified the cardiac defects seen in the previous work by this group. In the 500 ppm and 750 ppm concentration groups, a concentration-related increase in heart defects was found, including tricuspid valve, papillary muscle, and chordae tendineae abnormalities. Maternal neurotoxicity (ataxia, hypersensitivity to touch or sound, tremors, and convulsions) occurred in the 750 ppm group.

Rat reproductive studies have demonstrated testicular effects and/or decreased fertility with exposure levels >6195 mg/m³ chloromethane (Chapin et al., 1984; Chellman et al. 1987; Working and Bus 1986; Working et al. 1985a, b).

DERIVATION OF A PROVISIONAL SUBCHRONIC RfC

CIIT (1979) identified a NOAEL of 1530 mg/m³ and a LOAEL of 3041 mg/m³ for the following critical effects: increased liver enzymes, organ weight, and hepatocellular vacuolization. This study was used to derive the subchronic RfC. Calculation of the human equivalent concentration for the NOAEL is as follows:

- a. NOAEL of 1530 mg/m³ is adjusted for intermediate exposure:

$$\begin{aligned}\text{NOAEL}_{\text{ADJ}} &= 1530 \text{ mg/m}^3 \times 6 \text{ hours}/24 \text{ hours} \times 5 \text{ days}/7 \text{ days} \\ &= 273 \text{ mg/m}^3\end{aligned}$$

- b. Derivation of the NOAEL_{HEC}:

$$\begin{aligned}\text{NOAEL}_{\text{HEC}} &= 273 \text{ mg/m}^3 \times (\text{b:a } \lambda(\text{a})/\lambda(\text{h})) \\ &= 273 \text{ mg/m}^3\end{aligned}$$

where: NOAEL_{HEC} was calculated for a gas:extrarrespiratory effect, assuming periodicity was attained. As the mouse/human blood/gas partition coefficient ratio is unknown, a default ratio of 1.0 was assumed for calculation.

The NOAEL_{HEC} of 273 mg/m³ was divided by an uncertainty factor of 30 (10 to protect sensitive human subpopulations and 3 for interspecies extrapolation) to yield a provisional subchronic RfC of 9E+0 mg/m³.

The critical study (CIIT, 1979) is given medium confidence as it appears to be well-conducted using appropriate number of animals and exposure levels. Unfortunately, only a summary of the study was provided, and it does not appear that histopathological examination of the respiratory tract or all major organs was conducted. However, the NOAEL identified in this study is supported by hepatotoxicity seen in mice in the subchronic studies by McKenna et al. (1981a), and in mice at the 6 month interim sacrifice, prior to neurotoxic effects, in the CIIT study (1981). It is also supported by the less-than-subchronic studies of Morgan et al. (1982) and Landry et al. (1985) after both continuous and intermittent sacrifice. Therefore, the derived provisional subchronic RfC is given a high confidence rating. Additionally, the database is given a high confidence rating because there is a chronic inhalation study in two species supported by subchronic inhalation studies in several species, and because data are available on the developmental and reproductive effects of chloromethane.

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**Risk Assessment Issue Paper for:
Evaluation of the Carcinogenicity of
1,2,4-Trimethylbenzene (CASRN 95-63-6)**

Introduction

A TOXLINE literature search (1986-1991, oral RfD and cancer strategies) was conducted in October 1991. In addition, literature searches of TOXLINE (1991-1993, CAS number and chemical name, all cites), MEDLINE (1990-1993), HSDB, RTECS, and TSCATS were performed in May 1993. The only documents listed on the CARA databases (U.S. EPA, 1991, 1994c) are a Health Effects Assessment document (U.S. EPA, 1987a) and a Health Advisory (U.S. EPA, 1987b), which were consulted. Other sources of information that were consulted were IRIS (U.S. EPA, 1995a), the CRAVE Monthly Status Report (U.S. EPA, 1995b), the Drinking Water Regulations and Health Advisories list (U.S. EPA, 1994a), the HEAST (U.S. EPA, 1994b), and NTP Status Reports (NTP, 1993a,b). 1,2,4-Trimethylbenzene has not been the subject of an ATSDR toxicological profile.

Summary of Relevant Data

The only pertinent information located in the sources mentioned above was a study by Rivedal et al. (1992) who reported that 1,2,4-trimethylbenzene did not induce morphological transformation of Syrian hamster embryo cells in culture, or increase the transformation frequency induced by benzo[a]pyrene. In addition, 1,2,4-trimethylbenzene did not inhibit intercellular communication. 1,2,4-Trimethylbenzene has not been selected for carcinogenicity testing by the National Toxicology Program (NTP, 1993a,b).

Weight of Evidence

Based on no human or animal data, 1,2,4-trimethylbenzene can be assigned to U.S. EPA weight-of-evidence Group D: not classifiable as to human carcinogenicity (U.S. EPA, 1986).

Derivation of Oral Slope Factor and Inhalation Unit Risk

Agents assigned to weight-of-evidence Group D are regarded as unsuitable for quantitative risk assessment (U.S. EPA, 1986).

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**Risk Assessment Issue Paper for:
Derivation of a Provisional RfC for
Trimethylbenzene (1,2,4 and 1,3,5)
(CASRN 108-67-8 and 95-63-6)**

A TOXLINE literature search (1981-1991, oral RfD and cancer strategies) was conducted in November 1991. In addition, literature searches of TOXLINE (1991-1993, CAS number and chemical name, all cites), MEDLINE (1990-1993), CANCERLINE (mid-70's-1993, all cites), HSDB, RTECS, and TSCATS were performed in May 1993. Documents on the CARA lists (U.S. EPA, 1991, 1994c) are a Health Effects Assessment document (U.S. EPA, 1987a) and a Health Advisory (U.S. EPA, 1987b), which were consulted. Other sources of information that were consulted were IRIS (U.S. EPA, 1995a), the RfD/RfC Monthly Status Report (U.S. EPA, 1995b), the Drinking Water Regulations and Health Advisories list (U.S. EPA, 1994a), the HEAST (U.S. EPA, 1994b), and NTP Status Reports (NTP, 1993a). Trimethylbenzene has not been the subject of an ATSDR toxicological profile.

Summary of Relevant Toxicity Data for Trimethylbenzene

Data regarding the inhalation toxicity of trimethylbenzene are limited to one occupational study (Battig et al., 1958) and several acute and subchronic studies in rodents (Battig et al., 1958; Bernshtein, 1972; Cameron et al., 1938; Lazarew, 1929; Wiglusz et al., 1975a,b). Of all these studies, only Wiglusz et al. (1975a) was available for review; details of the others were obtained from secondary sources. Based on the available data, U.S. EPA (1987a) determined that the data were inadequate for derivation of a subchronic or chronic inhalation RfC.

The U.S. EPA (1987b,c) reported the results of the occupational study of Battig et al. (1958). In that study, an increase in toxic symptoms was found in 27 workers exposed for several years to "Fleet-X-DV-99", as compared to 10 unexposed controls. Fleet-X-DV-99 is a solvent containing 97.5% aromatic hydrocarbons (>30% 1,3,5-trimethylbenzene and >50% 1,2,4-trimethylbenzene) and 2.5% of paraffinic and naphthenic hydrocarbons. Rough quantitation of the exposure levels reported concentrations of hydrocarbons vapor ranging from 10 to 60 ppm (49-295 mg/m³). Clinical findings in the workers included central nervous system effects (vertigo, headaches, drowsiness), chronic asthma-like bronchitis (classification criteria not reported), hyperchromic anemia (<4.5 million erythrocytes/mm³) and disturbances in blood clotting.

A 4-hour inhalation LC₅₀ of 24 g/m³ was reported for rats (RTECS, 1993). U.S. EPA (1987b) summarized some acute data in rats and mice. Concentrations of 35-40 mg/L of 1,3,5-trimethylbenzene in the air (34,480-44,332 mg/m³) caused loss of reflexes and prostration in mice, but the duration of the exposures was not reported (Lazarew, 1929). Rats exposed to 2240 ppm (11,034 mg/m³) 1,3,5-trimethylbenzene for 24 hours slowly developed narcosis and 4/16 died from respiratory failure (Cameron et al., 1938). Congestion of the lungs was the only remarkable

pathology. No adverse effects were observed in rats and mice exposed to 560 ppm (2758 mg/m³) for 24 hours or to the same concentration 8 hours/day for 14 days (Cameron et al., 1938). Wiglusz et al. (1975a,b) reported an increase in the proportion of segmented neutrophilic granulocytes, and a decrease in the proportion of lymphocytes in rats exposed to 6.0 mg/L (6000 mg/m³) 1,3,5-trimethylbenzene for 6 hours. Serum alkaline phosphatase was increased at 3.0 mg/L and no adverse effects were noticed at 1.5 mg/L (1500 mg/m³).

U.S. EPA (1987a,b,c) reported the results of several subchronic inhalation studies in rats. Battig et al. (1958) exposed rats 5 days/week, 8 hours/day to 1700 ppm (8357 mg/m³) (8 rats) Fleet-X-DV-99 solvent for 4 months or to 500 ppm (2458 mg/m³) (unspecified number of rats) for 70 days. Four of the 8 rats exposed to 1700 ppm died within 2 weeks of exposure, while none of the animals in the 500 ppm group died. Histological changes in the animals that died (only these were examined) included cloudy swelling and fatty infiltration in the kidneys, peripheral fatty infiltration in the liver, an increase in secondary nodules in the spleen, and marked congestion of the pulmonary capillaries with alveolar wall thickening. Alterations in differential WBC counts (increase in the percentage of segmented neutrophilic granulocytes and a decrease in the percentage of lymphocytes) were reported at \geq 500 ppm. Similar alterations in differential WBC counts as well as a significant elevation of SGOT levels were found in rats (n=6) exposed to 3.0 mg/L (610 ppm) 1,3,5-trimethylbenzene 6 hours/day, 6 days/week for 5 weeks (Wiglusz et al., 1975a,b). No exposure-related effects on hemoglobin levels and erythrocyte or leukocyte counts, or on the activity of SGPT, glutamate dehydrogenase or ornithine carbamyl transferase were found in the Wiglusz et al. (1975a,b) studies. Bernshtein (1972) exposed rats to 1 mg/L (200 ppm) of a mixture of trimethylbenzenes 4 hours/day for 6 months. An inhibition of phagocytic activity of the leukocytes was reported. This study was summarized by Sandmeyer (1981) and further experimental details were not provided.

ACGIH (1986, 1992) has adopted a TLV-TWA of 25 ppm (123 mg/m³) for trimethylbenzenes based on the occupational study by Battig et al. (1958). OSHA (1992) lists an 8-hour TWA of 25 ppm for trimethylbenzenes to prevent adverse respiratory and blood effects in exposed workers (OSHA, 1989). NIOSH (1992) lists a REL TWA of 25 ppm to protect workers from skin irritation, CNS depression, and respiratory failure.

Derivation of a Provisional RfC for Trimethylbenzene

A provisional inhalation RfC can be derived for an isomeric mixture of trimethylbenzene based on the occupational study by Battig et al. (1958). Adverse respiratory, neurological, and hematological effects were reported in that study in workers exposed to a solvent containing >80% trimethylbenzenes. The lowest reported exposure concentration was 10 ppm (49 mg/m³, assuming the solvent content to be exclusively trimethylbenzenes) and constitutes a LOAEL. The RfC can be calculated as follows:

$$\begin{aligned} \text{RfC} &= 49 \text{ mg/m}^3 \times (10 \text{ m}^3/20 \text{ m}^3) \times (5 \text{ day}/7 \text{ day})/3000 \\ &= 17.5 \text{ mg/m}^3/3000 \end{aligned}$$

where: 49 mg/m³ = LOAEL

$10 \text{ m}^3/20 \text{ m}^3$ = adjustment assuming that occupationally exposed humans inhale $10 \text{ m}^3/\text{workday}$ and 10 m^3 during the remainder of the day.

5 day/7 day = adjustment for a 5-day work week.

3000 = uncertainty factor (10 for the use of a LOAEL, 10 to extrapolate from subchronic to chronic exposure, 10 to protect sensitive individuals, and 3 for incomplete database).

$$\text{RfC} = 6\text{E-}3 \text{ mg/m}^3$$

Confidence in the key study is low because a small number of subjects were examined, and the workers were exposed to other chemicals in the solvent mixture. Low confidence in the database reflects the lack of chronic, developmental or reproduction studies in animals by any route of exposure, and the poor quality of the available subchronic inhalation studies. Low confidence in the provisional inhalation RfC follows.

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