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Subchronic Inhalation Toxicity of Ethylbenzene in Mice, Rats, and Rabbits[1]

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*Radian Corporation, 13595 Dulles Technology Drive, Herndon, Virginia 22071; †Synthetic Organic Chemical Manufacturers Association, Washington, D.C. 20013; ‡Bio/dynamics, Inc., East Millstone, New Jersey 08873; §The Dow Chemical Company, Midland, Michigan 48640; ‖Hazleton Laboratories, Vienna, Virginia 22180; and ¶Hoechst-Roussel Pharmaceuticals Inc., Sommerville, New Jersey 08876

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Subchronic Inhalation Toxicity of Ethylbenzene in Mice, Rats, and Rabbits. CRAGG, S. T., CLARKE, E. A., DALY, I. W., MILLER, R. R., TERRILL, J. B., AND OUELLETTE, R. E. (1989). *Fundam. Appl. Toxicol.* **13**, 399–408. Mice, rats, and rabbits (five/sex/group) were exposed by inhalation to ethylbenzene (EB) vapors for 6 hr/day, 5 days/week for 4 weeks (20 exposures). Rats and mice received 0, 99, 382, or 782 ppm EB while rabbits received 0, 382, 782, or 1610 ppm. No changes were evident in mortality patterns, clinical chemistries, urinalyses, or treatment-related gross/microscopic (including ophthalmologic) lesions. Rats exhibited sporadic lacrimation and salivation, as well as significantly increased liver weights at 382 and 782 ppm, and small increases in leukocyte counts at 782 ppm. Males at this exposure level also showed marginal elevations in platelet counts. In mice, females showed statistically increased absolute and relative liver weights at 382 and 782 ppm, while males had statistically increased relative liver-to-brain weight ratios only at 782 ppm. Female rabbits at the high exposure level of 1610 ppm gained weight more slowly than controls (not statistically significant); males showed a similar transient downward trend after 1 week, but showed no differences from controls at study's end. A no observed adverse effect level (NOAEL) of 382 ppm appears appropriate for rats and mice with a lowest observed adverse effect level (LOAEL) of 782 ppm. A NOAEL of 782 ppm and LOAEL of 1610 ppm are appropriate for rabbits. © 1989 Society of Toxicology.

In 1986, U.S. production of ethylbenzene (EB) exceeded 8.5 billion pounds, 17th among all organic chemicals (Heylin, 1987). EB is used in the production of styrene and, to a more limited extent, as a solvent. Also occurring as a codistillate in various petroleum mixtures, EB comprises approximately 20% of commercial xylene and a small percentage of gasoline (mean concentration = 1.8%; range, 0.2 to 4.8%) (Toney, 1987). Xylene and gasoline represent significant additional annual EB production on the order of several billion pounds (Heylin, 1987).

Inhalation or dermal exposure to EB may occur during commercial xylene use or from pumping gasoline, but is minimal during EB-to-styrene conversion because the process is enclosed. Estimates of the number of individuals exposed range from thousands exposed to the pure chemical to hundreds of thousands exposed to xylenes and gasoline. In addition, the public has potential oral exposure to low levels (ppm) of EB from drinking groundwater contaminated with low boiling petroleum hydrocarbons. Currently, EB is regulated by a variety of federal laws, includ-

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ing the Clean Water Act (as a priority pollutant), the Safe Drinking Water Act, and the Resource Conservation and Recovery Act.

The acute toxicity and metabolic disposition of EB have been evaluated in several species (Sandmeyer, 1981). EB has low acute toxicity by the oral route [LD50 (rat) = 3500 mg/kg], by the dermal route [LD50 (rabbit) = 5000 mg/kg], and by inhalation [4 hr LC50 (rat) = 8000 ppm] (Sandmeyer, 1981; Smyth *et al.*, 1962). The human odor threshold for EB vapor is 10 ppm, and eye irritation occurs rapidly at 1000 ppm (Gerarde, 1960). Repeated human exposure to the liquid has resulted in dermatitis, presumably due to the lipid solubilizing properties of EB. The American Council of Governmental Industrial Hygienists recommends a threshold limit value (TLV) of 100 ppm and a short-term exposure limit of 125 ppm for EB. The current Occupational Safety and Health Administration permissible exposure limit is equivalent to the TLV.

The effects of repeated exposure to EB have not been as well-defined. Two subchronic inhalation studies have been conducted, both more than 20 years ago. Wolf *et al.* (1956) exposed rats, guinea pigs, rabbits, and monkeys to EB concentrations ranging from 400 to 2200 ppm for 7 to 8 hr/day, 5 day/week for 6 months. Only one or two animals per sex constituted an exposure group for rabbits and monkeys. Degeneration of the germinal epithelium of the testis, found in a single rabbit and monkey exposed to 600 ppm EB, has been widely cited in subsequent literature reviews (usually without acknowledgment that only a single male of each species was exposed and showed an effect) (ECETOC, 1986; Engstrom *et al.*, 1987; EPA, 1980, 1985, 1986; Haley, 1981). In a later study by Ivanov (1964), rabbits were exposed to 2.3, 23, and 230 ppm EB for 4 hr/day for 7 months. Further protocol details were not provided. The following dose-related changes were reported in that study: altered blood cholinesterase, decreased plasma albumin, increased globulin, leukocytosis, reticulocytosis, and dystrophic changes in the kidneys and liver.

The present study was undertaken to characterize the subchronic inhalation toxicity of EB to the mouse, rat, and rabbit. In order to select appropriate exposure concentrations, a 4-day range-finding study was conducted using concentrations that met or exceeded those in previous studies. Male mice, rats, and rabbits (four to five animals/species) were exposed to 400, 1200, and 2400 ppm EB for 6 hr/day. At 2400 ppm, EB caused 100% mortality in mice and rats within the 4-day treatment period. At 1200 ppm, four of five mice died while the surviving mouse and all rats showed marked clinical signs, including respiratory distress, lacrimation, salivation, prostration, and anogenital staining. No effects were noted in mice or rats at 400 ppm. All rabbits in each exposure group survived and showed no adverse signs.

Because of the lethality and severe distress observed at EB concentrations of 1200 ppm and higher in the range-finding study, 800 ppm was targeted as the highest exposure concentration for rats and mice in the primary study. Although rabbits tolerated 2400 ppm EB without ill effects, the maximum exposure level for this species was reduced from 2400 ppm to a target concentration of 1600 ppm in the main study to minimize technical difficulties encountered in generating the higher chamber concentration.

MATERIALS AND METHODS

Test material. EB was supplied by Dow Chemical with a purity of 99.7%. The major impurities were toluene (0.15%), isopropanol (0.04%), and benzene (0.005%).

Animals. Sixty-five male and female Fischer 344 rats (approximately 6 weeks old) and 65 male (aged 7 weeks) and female (aged 9 weeks) B6C3F1 mice were purchased from Charles River Breeding Laboratories, Inc. (Kingston, NY). Both species were acclimated for 17 days. Fifty New Zealand White rabbits were purchased from Hazleton-Dutchland, Inc. (Denver, PA) and were acclimated for 6 weeks. At the time of testing, 20 animals/sex/species were selected. Test animal weights ranged between 158 and 178 g (male rats); 124 and 138 g (female rats); 19 and

24 g (male mice); 17 and 21 g (female mice); 2934 and 3866 g (male rabbits); and 3034 and 3758 g (female rabbits). All animals were examined by a clinical laboratory veterinarian and were found to be in good health prior to study initiation.

Housing and maintenance. Rats and mice were housed two per cage in suspended stainless-steel wire mesh cages during the first week of acclimation and individually during all other nonexposure periods. Rabbits were individually housed during acclimation and nonexposure periods. All animals were housed individually in wire mesh stainless-steel cages during the treatment period. Access to food and water was provided *ad libitum* except during the 6-hr daily exposures. Mice and rats were given Purina Certified Rodent Chow No. 5002, and rabbits were fed Purina High-Fiber Rabbit Chow No. 5326. A 12-hr light/dark cycle was maintained each day. Temperature averaged 72 ± 3°F and humidity ranged between 30 and 70%.

Experimental design. Each exposure group consisted of five animals per sex. Rats and mice were exposed to 0, 99, 382, or 782 ppm EB vapors while rabbits received exposures of 0, 382, 782, or 1610 ppm. Species were housed together in common chambers during exposures and apart during nonexposure periods. Rats and rabbits, but not mice, were subjected to clinical laboratory studies. All animals underwent necropsy at the end of the exposure period and tissues from all were saved. The high-exposure and control animals (of all species) underwent histopathological evaluation; the testes of low- and mid-exposure rabbits were also examined.

Chambers. Exposures to EB vapor were conducted in 10-m^3 stainless-steel and glass chambers. Within the chambers, animals were housed individually in suspended stainless-steel wire mesh cages. Total animal body volume did not exceed 5% of chamber volume. Chamber flow rate averaged 3000 liters/min, or 18 air changes/hr.

Vapor generation and analysis. FMI fluid metering pumps (Model No. RPG-20) with either a $\frac{1}{4}$- or $\frac{3}{8}$-in. piston were used to meter EB into an air atomizing nozzle (Spraying Systems C., $\frac{1}{4}$-in. JSS nozzle with a No. 1A spray setup). House-supplied air was introduced into the atomizer at 20 psi to generate an aerosol that immediately volatilized to the vapor state. A TSI aerodynamic particle sizer (Model No. 3300) with a TSI No. 3302 diluter was used once per exposure period to determine whether EB was in the vapor phase. Readings indicated that less than 0.1% EB was present as aerosol at any time.

EB concentrations in each chamber were monitored at hourly intervals, six times per day with a Miran 1A (Foxboro Co.) infrared gas analyzer. Concentrations (mean daily time-weighted average) and standard deviations are reported in Table 1.

Actual analytical concentrations agreed well with nominal concentrations (the latter were calculated by dividing air flow rate into EB consumption rate). Actual concentrations were 91% of nominal concentrations for Group 2 and >95% for the remaining groups. Chamber concentrations varied less than 10% of the mean for more than 90% of the exposure days, and actual concentration was within approximately 5% of the target concentration for each exposure chamber.

Criteria of response. Animals were observed twice daily for obvious clinical signs and underwent detailed examinations once each day after treatment. Body weights were measured weekly, and the weights of the major organs were recorded at all terminations. Ocular examinations were conducted by a board-certified veterinary ophthalmologist and were conducted on all animals immediately prior to exposure and at terminations. Atropine was used to induce mydriasis for ophthalmic examinations, in which the cornea, anterior chamber, lens, iris, vitreous humor, retina, and optic disc were subsequently examined by indirect ophthalmoscopy. In addition, conjunctiva and lids were examined grossly.

Blood was collected via venipuncture of the orbital sinus (retrolobular venous plexus, under light ether anesthesia) for rats and mice and from the medial artery of the ear for rabbits. Blood samples from all animals were subjected to hematological evaluation on a laser hematology counter ELT-8 (Ortho Instruments), while blood chemistries were evaluated only in rats and rabbits. Mice were not subjected to clinical chemistry examinations because the volume of blood that could be collected for this purpose was too small for evaluation without pooling (which would have reduced statistical sensitivity). For similar reasons, urinalyses were performed for rats but not mice. Rabbits were also excluded from urinalysis for logistical reasons. Hematology was performed on all species and parameters included hematocrits, hemoglobin, erythrocyte counts and morphology, platelet counts, and differential white blood cell counts. Clinical chemistry parameters were measured automatically on a Centrifi-Chem System 500. These parameters included glucose, blood urea nitrogen, total protein, albumin, A/G ratio, calcium, creatinine, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, alkaline phosphatase, and lactic dehydrogenase. Urine was collected using metabolism pans attached to individual animal cages. Urine samples were examined for color and appearance, pH, glucose, ketones, urobilinogen, occult blood, volume, specific gravity, protein, and bilirubin.

At termination, rats and mice were exsanginated under ether anesthesia and rabbits were subjected to an overdose of sodium pentobarbitol. Over 30 tissues from each of the high-exposure and control animals of all species were subjected to histopathological examination. These included abdominal aorta, adrenals, bone and bone marrow (sternum), brain, esophagus, eyes, femur, gall bladder (mice and rabbits only), gonads (ovaries or testes with epididymides), heart, intestine (cecum, colon, duodenum, ileum, jejunum, and rectum), kidneys, liver, lungs, lymph nodes, nasopharyngeal tissues, pancreas, pituitary, salivary glands, sciatic nerve, spleen, stomach,

TABLE 1

ETHYLBENZENE CHAMBER CONCENTRATIONS (MIRAN 1A INFRARED ANALYZER MEASUREMENTS)

Group	Target concentration (ppm)	Nominal concentration (ppm ± SD)	Actual concentration (ppm ± SD)	Coefficient of variation (actual)
1	0	0	0	—
2	100	109 ± 2	99 ± 3	3.0%
3	400	393 ± 12	382 ± 20	5.2%
4	800	812 ± 20	782 ± 28	3.6%
5	1600	1650 ± 104	1610 ± 82	5.1%

Note. Nominal concentrations were calculated as weight of material used per unit volume of air and expressed as the equivalent parts per minute concentration. Actual concentrations were made with a Miran 1A at hourly intervals each day (see narrative for further details).

thymic region, thyroid/parathyroid, trachea, urinary bladder, uterus (horns and cervix), gross lesions, tissue masses or suspect tumors, and regional lymph nodes. In addition, testicular tissues from the low- and intermediate-exposure rabbits were also examined. Tissues from all exposure levels were preserved (fixed in 10% neutral buffered formalin) in the event that histopathological lesions were found in the high-exposure group. Tissues undergoing histopathological examination were embedded in paraffin, cut at 6 μm, and mounted and stained with hematoxylin and eosin.

Statistics. All parameters were evaluated for homogeneity of variance by Bartlett's test. When homogeneous, analysis of variance was conducted using the F distribution to assess significance. If the overall F statistic was significant, Dunnett's test was used to compare specific treatment groups to the control. The nonparametric Kruskall–Wallis test was used when variances were heterogeneous, and Dunn's summed-rank test was used to compare treated groups to controls.

RESULTS

Clinical signs. No animals died during the course of exposure. Rats exposed to 382 and 782 ppm EB showed sporadic incidences of salivation and lacrimation that may be treatment-related, although the frequencies were insufficient to determine a dose–response relationship. No other signs were considered treatment-related.

Body weights. EB-exposed rats and mice of both sexes showed normal weight gains during the course of exposures. In rabbits, male and female controls showed less weight gain during the first week when compared to their gains over the remainder of the study, while four of the six exposure groups showed slight-to-moderate weight loss (Table 2). This trend was more obvious in females than in males. Throughout the study, weekly weight gains in females were smaller in EB-exposed groups while differences in body weight were not statistically different from controls. At the end of exposures, differences in body weight were still evident but not statistically significant for females (Table 3). After the first week of treatment, male rabbits in the exposed groups gained weight at the same rate as controls and had comparable body weights at the end of treatment.

Organ weights. Rats and mice of both sexes generally exhibited statistically significant increases in mean liver weights, liver-to-body weight ratios, and liver-to-brain weight ratios in the 782-ppm groups (Tables 4 and 5). Male and female rats exposed to 382 ppm EB showed similar but less marked increases in these three parameters that generally were not significant. EB concentrations of 99 ppm produced no changes. Rabbits showed no changes in liver weights/ratios at any exposure level. No other organ weights were changed.

Hematology. Small but statistically significant increases in platelet counts occurred in male rats exposed to 782 ppm EB. In the

TABLE 2

RABBIT BODY WEIGHTS IN GRAMS (± SD)

Group	ppm	N	Pretest	Week 1	Week 2	Week 3	Week 4
				Males			
I	0	5	3260 ± 296	3288 ± 253	3402 ± 318	3492 ± 324	3560 ± 302
III	382	5	3288 ± 362	3280 ± 290	3368 ± 361	3439 ± 400	3549 ± 354
IV	782	5	3302 ± 182	3340 ± 184	3447 ± 224	3575 ± 255	3668 ± 254
V	1610	5	3278 ± 201	3265 ± 161	3352 ± 204	3457 ± 194	3543 ± 219
				Females			
I	0	5	3343 ± 201	3361 ± 81	3492 ± 108	3612 ± 128	3751 ± 197
III	382	5	3419 ± 198	3425 ± 181	3643 ± 221	3749 ± 202	3943 ± 286
IV	782	5	3448 ± 226	3401 ± 186	3545 ± 192	3640 ± 167	3800 ± 184
V	1610	5	3317 ± 215	3255 ± 227	3390 ± 263	3476 ± 271	3561 ± 301

high-exposure group, male rats showed a marginal increase and female rats a statistically significant increase in mean total leukocyte counts. These results are shown in Fig. 1–3. The leukocytosis did not not notably affect the differential white cell count (which was not subject to statistical analysis). No other hematological parameters were altered for any of the species.

Clinical chemistry. No alterations were noted in rats or rabbits exposed to EB at any dose level. As previously explained, mice were not subjected to clinical chemistry examinations.

TABLE 3

RABBIT ABSOLUTE AND RELATIVE BODY WEIGHT GAIN[a]

Group	ppm	Weight gain (g) Males (%)	Weight gain (g) Females (%)
I	0	300 (9.2%)	408 (12.2%)
III	382	261 (7.9%)	524 (15.3%)
IV	782	365 (11.0%)	352 (10.2%)
V	1610	265 (8.1%)	244 (7.4%)

[a] Absolute weight gain was determined by subtracting body weight at termination from baseline body weights. Relative body weights were derived by dividing absolute weight gains by baseline body weights.

Urinalysis. Rats, the only species subjected to urinalysis, exhibited no unusual findings.

Gross and microscopic pathology. No gross or microscopic changes attributable to EB exposure were noted in any of over 30 tissues from each of the three species exposed to EB vapors at the high concentration. Tissues from low- and mid-dose animals were not examined except for rabbit testes (no abnormalities were found).

DISCUSSION

In summary, EB caused several changes which were marginal in degree or of questionable clinical significance when rats and mice were exposed to concentrations as high as 782 ppm and when rabbits were exposed to concentrations of 1610 ppm for 28 days (20 exposures). Specifically, rats and mice exhibited liver weight and weight ratio increases. Rats also exhibited sporadic lacrimation and salivation, and, at 782 ppm, males exhibited slight but significantly elevated platelet counts while females exposed to this concentration showed slight but significantly elevated leukocyte counts. Rabbits exhibited a transient but nonstatistically significant reduction in body weight when treated with EB at 1610 ppm.

TABLE 4

LIVER WEIGHTS AND RATIOS (MALES)

	Exposure concentration (ppm)	Grams body weight (SD)	Grams liver weight (SD)	Liver-to-body weight ratio (×100) (SD)	Liver-to-brain weight ratio ratio (SD)
Male mice	0	19 (1)	1.00 (0.08)	5.16 (0.21)	2.36 (0.23)
	99	22 (2)	1.12 (0.11)	5.15 (0.25)	2.47 (0.17)
	382	20 (1)	1.10 (0.09)	5.53 (0.24)	2.51 (0.18)
	782	22 (2)	1.20 (0.15)	5.52 (0.41)	2.77 (0.20)*
Male rats	0	206 (15)	5.99 (0.35)	2.91 (0.10)	3.34 (0.15)
	99	214 (10)	6.32 (0.19)	2.95 (0.08)	3.49 (0.11)
	382	217 (7)	6.73 (0.33)*	3.10 (0.07)	3.67 (0.16)
	782	208 (12)	6.82 (0.65)*	3.28 (0.18)**	3.77 (0.36)*
Male rabbits	0	3481 (316)	95.7 (20)	2.70 (0.40)	10.1 (2.09)
	382	3437 (326)	87.0 (16)	2.50 (0.40)	9.31 (1.79)
	782	3599 (245)	91.1 (11)	2.50 (0.30)	9.22 (1.41)
	1610	3452 (234)	92.7 (18)	2.70 (0.40)	9.51 (1.29)

* $p < 0.05$.
** $p < 0.01$.

The most marked effect from EB exposure was the statistically significant increase in liver weights and ratios observed in mice and rats. The absence of accompanying liver histopathology or abnormal clinical chemistry indicates that the increases were due to an adaptive induction of hepatic mixed function oxidase rather than toxicity. This conclusion

TABLE 5

LIVER WEIGHTS AND RATIOS (FEMALES)

	Exposure concentration (ppm)	Grams body weight (SD)	Grams liver weight (SD)	Liver-to-body weight ratio (×100) (SD)	Liver-to-brain weight ratio ratio (SD)
Female mice	0	19 (1)	0.92 (0.06)	4.97 (0.31)	2.01 (0.13)
	99	19 (0)	0.95 (0.03)	4.94 (0.13)	2.05 (0.07)
	382	19 (1)	0.99 (0.10)	5.17 (0.33)	2.20 (0.20)
	782	20 (1)	1.06 (0.04)*	5.30 (0.09)	2.32 (0.08)**
Female rats	0	141 (6)	4.12 (0.24)	2.93 (0.07)	2.37 (0.12)
	99	142 (6)	4.24 (0.26)	2.98 (0.14)	2.47 (0.12)
	382	139 (7)	4.36 (0.30)	3.14 (0.15)*	2.51 (0.09)
	782	145 (3)	5.11 (0.13)**	3.52 (0.10)**	2.98 (0.12)**
Female rabbits	0	3729 (213)	80.8 (11)	2.16 (0.26)	8.91 (1.48)
	382	3888 (273)	94.1 (21)	2.41 (0.42)	9.83 (1.94)
	782	3726 (172)	79.3 (9.6)	2.13 (0.22)	8.35 (1.12)
	1610	3634 (276)	81.9 (12)	2.25 (0.21)	8.35 (1.43)

* $p < 0.05$.
** $p < 0.01$.

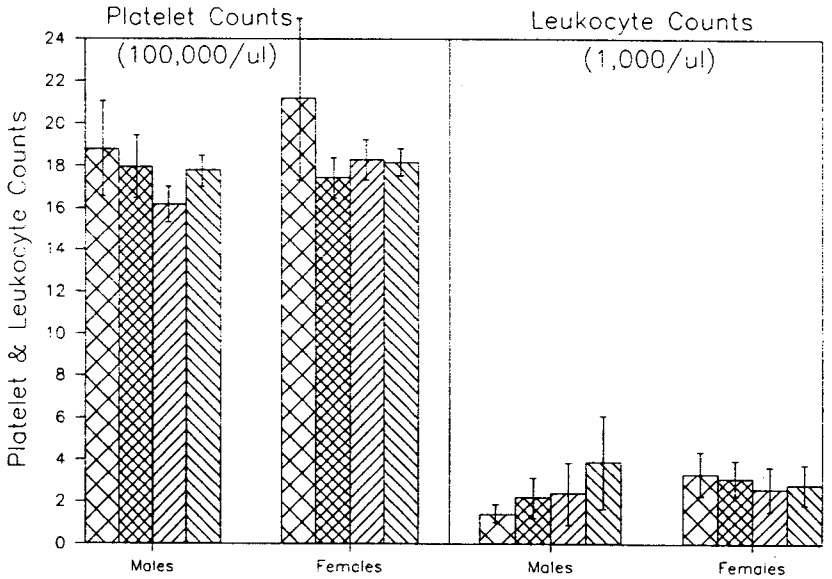

FIG. 1. Mouse hematology. ▩ 0 ppm; ▩ 99 ppm; ▨ 382 ppm; ▨ 782 ppm. Bar heights represent group mean values and brackets define standard deviation.

is further supported by the investigations of Toftgard and Nilsen (1982) and Elovaara *et al.* (1985), which have shown that rat cytochrome P450 is induced by inhalation exposure to comparable concentrations of EB.

Except for the liver weight changes, most

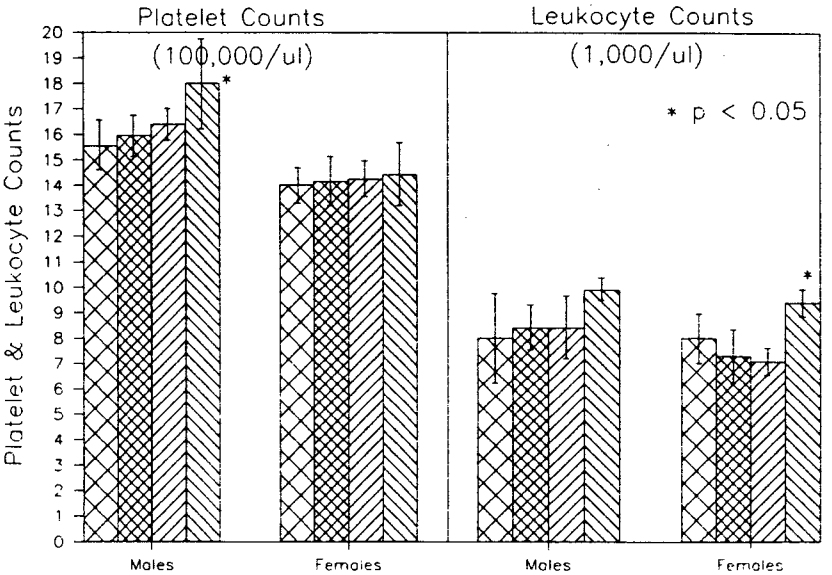

FIG. 2. Rat hematology. ▩ 0 ppm; ▩ 99 ppm; ▨ 382 ppm; ▨ 782 ppm. Bar heights represent group mean values and brackets define standard deviation.

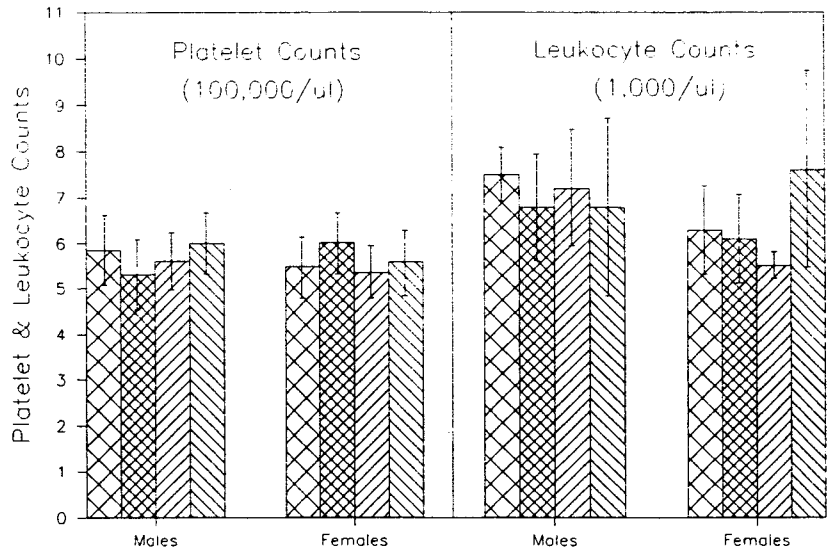

FIG. 3. Rabbit hematology. ▨ 0 ppm; ▩ 382 ppm; ▨ 782 ppm; □ 1610 ppm. Bar heights represent group mean values and brackets define standard deviation.

of the foregoing responses were marginal. No pattern was evident among the changes that might point to an underlying toxic response. Moreover, the various changes observed in the rats did not occur within the same animal (e.g., the rat with the highest leukocyte count was not the same animal with the highest platelet count, largest absolute/relative liver weight, or incidence of salivation or lacrimation). Finally, because this study monitored many toxicity parameters for each species and sex, it is possible that some have fallen outside the 95% confidence limits due to normal statistical variability (yielding a statistically significant p value that may not be biologically significant).

Overall, exposure to vapors of EB did not cause significant toxicity at the concentrations employed in this study. A no observed adverse effect level (NOAEL) of 382 ppm for rats and mice is warranted since the only statistically significant effect found at this level was a slight increase in liver weights for rats which was not consistent between the sexes. A lowest observed adverse effect level (LOAEL) may be set at the next highest exposure concentration of 782 ppm. At this level, liver weights and ratios were more consistently and markedly altered, although the "adverse" nature of this finding is debatable. The increased platelet and leukocyte counts in the rats at 782 ppm EB would also argue for a LOAEL at this concentration although the clinical significance of the latter findings is also questionable.

In rabbits, a NOAEL of 782 ppm may be appropriate, reflecting the transient initial decrease in body weights for the high-dose females during the first week of exposure. However, none of the body weight changes, even at the high concentration of 1610 ppm (a conservative LOAEL), were statistically significant.

The general lack of toxicity found in this 28-day study supports the hypothesis that EB is not a cumulative toxin, which is consistent with the rapid metabolism of this compound (EPA, 1986). In the range-finding study, exposure of mice and rats to 2400 ppm resulted in 100% mortality and 1200 ppm caused marked clinical signs in mice and rats and mortality in four of five mice. As 782 ppm EB in this study produced only minor toxicity, this suggests a mortality threshold for mice

somewhere near 1000 ppm and a steep dose–response curve. In rats, the mortality threshold is slightly higher (since rats were severely distressed but survived 1200 ppm EB); however, the dose–response curve is steep in this species as well given the relative absence of toxicity at 782 ppm. Rabbits did not appear to be significantly affected by any of the EB concentrations used in these studies.

Curiously, the Toftgard and Nilsen study (1982) induced cytochrome P450 in rats by inhalation of 2000 ppm EB vapors for 6 hr/day over 3 consecutive days without apparent toxicity (no mortality or adverse signs were reported).

Explanations for this apparent difference in toxicity might include strain differences and/or chamber atmosphere generation/analysis techniques (EB purity does not appear to be a factor, as both investigations used EB of greater than 99% purity). Sprague–Dawley rats were used in the Toftgard study while Fischer 344s were used in this investigation. Aerosolization was used in this study and, apparently, evaporation was used in the Toftgard study. When addressing inhalation procedures, the Toftgard paper referred to a second publication (Toftgard *et al.* 1981) which indicated only that saturated atmospheres of EB were mixed with air to achieve the desired concentrations (whether generated by aerosolization or evaporation was not indicated although evaporation seems likely).

Recent inhalation studies at Bushy Run (Pittsburg, PA) and other laboratories have shown a markedly higher inhalation toxicity for certain proprietary compounds when exposure atmospheres have been generated by aerosolization as opposed to evaporation from a high surface area medium (D. R. Klonne, personal communication). It has been hypothesized that microaerosols may exist (even though not detectable with a TSI particle sizer), which enhance deposition within the lung. Alternatively, evaporation may leave behind a toxic, higher boiling component which aerosolization would not. The latter possibility seems unlikely, at least for the proprietary chemicals tested to date, since extensive chemical characterization of the residues (when there are any) left on the evaporation surface does not reveal the presence of toxic constituents. Resolution of the apparent differences in toxicity observed in this and the Toftgard study await further investigation where the variables of animal strain and chamber generation methods can be controlled.

The oft-cited testicular toxicity reported by Wolf *et al.* (1956) in rabbits and monkeys (actually found in only a single exposed male of each species) exposed to EB vapors for 20 weeks was not detected in this study. Although the duration of exposure was 4 weeks in the present study, rabbits were subjected to substantially higher EB concentrations. Specifically, male rabbits exposed to 782 ppm ($N = 5$) and 1610 ppm ($N = 5$) EB vapors did not show the testicular changes found in the single rabbit and monkey exposed to 600 ppm in the Wolf study. This suggests that the observation of slight degenerative changes in the germinal epithelium of the male rabbit and monkey from the Wolf study may not be related to EB exposure. This is plausible in view of the high background incidence of testicular changes in the case of rabbits, particularly when they are stressed (Morton, 1988).

On the basis of the results of this study, it may be concluded that ethylbenzene is not highly toxic by the inhalation route and that most of the effects found were marginal, of questionable clinical significance, and/or not found with consistency between the sexes or among species. This is true for the slight elevations in platelet and leukocyte counts in the rats, body weight changes in female rabbits, and signs of lacrimation and salivation in rats. The marked liver weight and weight ratio increases found in this study, occurring without accompanying histopathology, support the conclusion that EB has induced hepatic mixed function oxidase as has been found directly by other investigators. Results of this study indicate that a subchronic inhalation LOAEL of 782 ppm and a NOAEL of

382 ppm are appropriate for rats and mice, while a LOAEL of 1610 ppm and a NOAEL of 782 ppm are appropriate for rabbits.

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