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Two-Generation Reproductive Toxicity Study of Inhaled Toluene Diisocyanate Vapor in CD Rats

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Twenty-eight 42-day-old pups/sex/group (F0) were exposed to toluene diisocyanate vapor (TDI; 80% 2,4-TDI, 20% 2,6-TDI) by inhalation at 0.0, 0.02, 0.08, or 0.3 ppm, 6 h/day, 5 days/week, for 10 weeks, then mated within groups for 3 weeks, with exposure 7 days/week during mating, gestation, and lactation. F0 maternal animals were not exposed from gestational day (gd) 20 through postnatal day (pnd) 4; maternal exposures resumed on pnd 5. Twenty-eight weanlings/sex/group continued exposure for 12 weeks (starting on pnd 28) and were bred as described above. F0 and F1 parents and ten F1 and F2 weanlings/sex/group were necropsied, and adult reproductive organs, pituitary, liver, kidneys, and upper respiratory tract (target organs) were evaluated histologically in ten/sex/group. Adult toxicity was observed in both sexes and generations at 0.08 and 0.3 ppm, including occasional reductions in body weights and weight gain, clinical signs of toxicity at 0.08 and 0.3 ppm, and histologic changes in the nasal cavities at 0.02, 0.08, and 0.3 ppm (including rhinitis, a nonspecific response to an irritating vapor, at all concentrations). There was no reproductive toxicity, reproductive organ pathology, or effect on gestation or lactation at any exposure concentration. Postnatal toxicity and reduced body weights and weight gains during lactation occurred only in F2 litters at 0.08 and 0.3 ppm. Therefore, under the conditions of this study, a no observed adverse effect level (NOAEL) was not determined for adult toxicity; the NOAEL for reproductive toxicity was at least 0.3 ppm, and the NOAEL for postnatal toxicity was 0.02 ppm.

Key Words: **toluene diisocyanate; two-generation study; parental toxicity; offspring toxicity; rhinitis; vapor exposure.**

Toluene diisocyanate (TDI) is used in the manufacture of polyurethane plastics. It is recognized as a respiratory sensitizer, and occupational exposure standards reflect the degree of control that is important to prevent respiratory effects such as occupational asthma. The American Conference of Government Industrial Hygienists (ACGIH) threshold limit value (TLV) for TDI is 0.005 ppm for an 8-h TWA, and the short-term exposure limit (STEL) is 0.002 ppm (ACGIH, 1992). Epidemiologic studies have been conducted that evaluate the low level of inhalation exposure to TDI in TDI production facilities (Diem *et al*., 1982; Musk *et al*., 1982; Olsen *et al*., 1989) and TDI-based foam manufacturing (Schnorr *et al*., 1996), with respiratory tract and skin sensitization effects typically not observed at exposure concentrations < 0.02 ppm. These epidemiologic studies have been used by the ACGIH to establish the need for preventing excessive exposure to TDI and to support the current guidelines (ACGIH, 1992).

Studies in laboratory animals have confirmed the potential for respiratory sensitization (Karol, 1983). In an inhalation carcinogenicity bioassay by Loeser *et al*. (1983), groups of rats and mice were exposed to 0, 0.05, and 0.15 ppm TDI for six h/day, 5 days/week, for approximately 2 years. There was no carcinogenic effect, though there were indications of irritation of the respiratory tract. In a chronic oral toxicity study, rats and mice were administered commercial grade TDI mixed in corn oil by gavage for 5 days/week for 105–106 weeks. Doses were 60 or 120 mg/kg for female rats and female mice; 30 or 60 mg/kg for male rats; and 120 or 240 mg/kg for male mice. Under these conditions, TDI in corn oil was considered to be carcinogenic for Fischer 344/N rats (National Toxicology Program, [NTP], 1986).

Chronic dietary administration of 2,4-toluenediamine (TDA), a metabolite of TDI, has been associated with increased tumor incidence in rats and female mice (NCI, 1979). However, the formation of TDA as a metabolic by-product following exposure to TDI appears to be dependent on the route of TDI exposure. Inhalation exposure to 2,4-TDI primarily resulted in the formation of acid labile conjugates with little or no 2,4-TDA being formed. When administered orally, however, substantially more TDA derivatives were found in urine, and some free TDA was measured (Timchalk *et al*., 1994). Therefore, the gavage route of administration, using high doses of TDI in corn oil, is not likely to be relevant to the potential carcinogenic risk to humans to TDI because of TDI's chemical

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reactivity and route-specific biochemical metabolism. Holder and Greenberg (1997) recognized the potential of inhaled TDI toxicity to the respiratory tract, but considered that TDI was not likely be a carcinogen by the inhalation route; Doe and Hoffmann (1995) concluded that low vapor exposures at current occupational standards would pose no significant carcinogenic risk.

A developmental toxicity study of commercial grade TDI was performed in rats, with the route of administration by whole-body inhalation of the vapor during major organogenesis (gd 6 through 15), 6 h per day. This study confirmed effects of TDI on the maternal respiratory tract, with concomitant reductions in maternal body weight, weight gain, and feed consumption at 0.50 ppm. There was only one indication of possible minor fetotoxicity at 0.50 ppm (increased incidence of reduced ossification in cervical centrum no. 5), with no other indications of developmental toxicity. There was no evidence of maternal or developmental toxicity at 0.02 or 0.10 ppm, and no evidence of embryotoxicity or teratogenicity at any exposure concentration evaluated in that study (Tyl *et al.*, 1999).

There were no studies evaluating the potential reproductive toxicity of TDI administered by inhalation of the vapor, the most relevant route of occupational exposure, in animal models. Therefore, this study was performed in rats administered commercial grade TDI by inhalation over two generations, one litter per generation.

MATERIALS AND METHODS

Chemical and generation of atmospheres. Toluene diisocyanate test material [TDI; Benzene, 1,3-diisocyanatomethyl; C9H6N2O2; CAS No. 26471–62–5 (generic number for a mixture of 2,4- and 2,6-TDI)], a commercial grade of an 80:20 mixture of the 2,4- and 2,6-isomers (CAS Nos. 584–84–9 and 91–08–7, respectively), was received from Dow Chemical USA (Freeport, TX). The purity and stability of the test chemical were verified by analysis before, during, and after the study. The test material remained over 99% pure throughout the study. However, there were modest differences between the TDI isomer ratio in the test atmospheres as compared to the test material (see Results).

Generation of atmospheres and chamber conditions. Target exposure concentrations for the exposure chambers were 0.0, 0.02, 0.08, and 0.3 ppm. The rationale for these target exposure concentrations was based on a developmental toxicity study of TDI at 0.02, 0.10, and 0.50 ppm in the same rat strain (Tyl, 1988; Tyl *et al.*, 1999). In that study, exposure to TDI vapor for six h/day, for gd 6 through 15 at 0.50 ppm, resulted in reductions in maternal body weight, body weight gain, and feed consumption. Because relatively major maternal indications of toxicity were observed at 0.50 ppm after only 10 days of exposure during gestation, there was concern that 0.50 ppm would result in excessive adult toxicity during the 10-week prebreed exposure and during the mating, gestation, and lactation periods (when exposure continued). Therefore, the top and middle target exposure concentrations were slightly lower for the present study.

The description of the inhalation exposure chambers, chamber airflow, illumination, and chamber temperature and relative humidity readings were as described by Tyl *et al.* (1999). Within each chamber, animal cages were rotated weekly to compensate for any possible variation in chamber exposure conditions (i.e., vapor concentration, temperature, or relative humidity).

TDI vapor was generated using a glass evaporator system similar in design to that described by Carpenter *et al.* (1975) and as described by Tyl *et al.* (1999). Temperature measurements obtained from the inside surface of each evaporator during the exposure regimen using a Doric Trendicator Model 400A probe (Doric Scientific Division, San Diego, CA) ranged from 42 to 63°C.

Two Autostep Isocyanate paper tape monitoring devices (GMD Systems, Inc., Hendersonville, PA), one for 0.00, 0.02, 0.08, and one for 0.3 ppm, were used to measure TDI concentrations in the exposure chamber atmospheres. Both instruments, calibrated using a modified Marcali method for detection of TDI in air, were equally sensitive to detection of the 2,4- and 2,6-TDI isomers. Throughout the 235 days of exposure, generated TDI atmospheres were monitored by placing probes in the breathing zone of the animals approximately six times per each 6-h exposure. Control chamber atmospheres were measured six times daily for the first 11 exposure days and once per day thereafter.

The 2,4- and 2,6-TDI isomer concentrations in the exposure chamber atmospheres were measured prior to the onset of the F0 exposure period and on exposure day 143. Samples were collected using glass fiber filters coated with 1-(-pyridylpiperazine), as well as impingers containing N-2-nitrobenzyl-n-m-propylamine in toluene. Samples were obtained through a sampling port located approximately 6 feet above the chamber floor and approximately 6 in. from the inside chamber wall. Probes for sampling were located within 2–3 in. of each other. Reverse phase high pressure liquid chromatography was used to separate and quantify the 2,4- and 2,6-isomers.

Animals and husbandry. One hundred forty-five virgin female and 145 virgin male albino CD® (Sprague-Dawley) rats (Crl:CD® [SD]BR) were received from Charles River Breeding Laboratories (Kingston, NY) and were 28 days of age upon arrival. These animals were quarantined for approximately 2 weeks, during which time they were weighed, examined by a veterinarian, and representative animals were subjected to fecal examination, serum viral antibody analysis, and histologic examination of selected organs. Results of the physical examination, serology, parasitology, and histopathology were negative for signs of infectious disease. Rats were housed initially two/same sex during quarantine and then singly, except for the cohabitation periods, for the duration of the study. During the quarantine, prebreed periods, mating, and most of gestation, rats were housed in stainless steel, wire mesh cages including during exposure periods. From gd 19 through parturition, and lactation until weaning, female rats were housed in plastic shoebox cages (19.0 × 10.5 × 8 in.) with Alpha-Dri® (Shepherd Specialty Papers, Inc., Kalamazoo, MI) bedding during nonexposure periods.

Feed (Certified Ground Rodent Chow® #5002, Ralston-Purina Company, St. Louis, MO) and tap water (Municipal Authority of Westmoreland County, Greensburg, PA) were available *ad libitum* except during exposures. The water was delivered by an automatic watering system, with demand control valves mounted on each cage rack for rats in stainless steel cages. Female rats housed in shoebox cages received water by water bottles with stainless steel sipper tubes. Analyses of the feed and water for contaminants and of the feed for nutrient levels indicated all contaminant levels were below the maximum certified standards, and all nutrient levels were above the minimum certified standards. Deotized Animal Cage Board® (Shepherd Specialty Papers, Inc., Kalamazoo, MI) was placed beneath the stainless steel cages and changed regularly. Animals were kept on a 12-h photoperiod, and room temperature and relative humidity were recorded continuously (Cole-Parmer Hygrothermograph® Seven-Day Continuous Recorder, Model No. 8368–00, Cole-Parmer Instrument Company, Chicago, IL). Temperature and relative humidity were maintained at 62–76°F and 40–70% in the animal rooms throughout the study (except for a few brief and/or minor excursions); light cycle was 12:12 h, and all animals were handled and treated at all times in conformance with the National Institutes of Health Guide (NIH, 1985).

Two hundred twenty-four animals were placed on study, 28/sex/group, by randomization procedures stratified by body weight, immediately prior to the start of the prebreed exposure period. All animals were assigned a unique number and were toe clipped and ear notched prior to the start of the study.

Study design. Twenty-eight 42-day-old rats (designated F0 generation) per sex per dose were exposed to TDI vapor by inhalation, 5 days/week, 6 h/day, for 10 weeks (prebreed exposure period), with clinical observations recorded daily (mortality checks twice daily) and body weights recorded weekly. Feed jars were changed weekly; feed consumption was not recorded.

Each female F0 animal was mated with a single male from the same exposure group for 3 weeks (with no change in partners), with exposure to TDI continuing throughout the mating period, 7 days/week. During the mating period, mating pairs remained together during the daily exposure periods. Observations of vaginal sperm and/or dropped or vaginal copulation plug were considered evidence of successful mating (Hafez, 1970); the date of insemination was designated gd 0. Once the animals mated, they were housed individually. Dams were weighed on gd 0, 7, 14, and 21. Exposure was continued throughout gestation and lactation for 6 h/day, 7 days/week, except for the following interval: after the exposure on gd 19, each female was transferred to a shoebox cage and was not exposed from gd 20 through pnd 4. Exposures to the dam resumed on pnd 5. The date of parturition was designated pnd 0. Dams with litters were weighed on pnd 1, 4, 7, 14, and 21. Pups (F1 generation) were individually counted, sexed, and examined grossly on pnd 0, 1, 4, 7, 14, and 21. They were also weighed individually on pnd 1, 4, 7, 14, and 21. Litters were randomly culled to a maximum of eight (with as equal sex ratio as possible) on pnd 4. At weaning on pnd 21, 28 F1 weanlings/sex/group were randomly selected to be parents of the F2 generation; ten F1 weanlings/sex/group were randomly selected for necropsy; remaining F1 weanlings were examined externally, euthanized, and discarded. Parental F0 animals were necropsied: males after the delivery of F1 litters, females after weaning of the F1 litters. After weaning on pnd 21, litters (minus the dam) were maintained for one additional week (until pnd 28) before direct exposures began to the selected F1 weanlings.

F1 selected weanlings were exposed to TDI by inhalation for 12 weeks, then mated as described above, with exposure continuing during mating, gestation, and lactation. F1 dams and F2 litters were handled as described above for F0 dams and F1 litters. F1 males were terminated after delivery of F2 litters; F1 females were terminated after their F2 litters were weaned. F2 weanlings, ten/sex/group, were selected for necropsy; remaining F2 weanlings were examined externally, euthanized, and discarded.

Necropsy and histopathology. All F0 and F1 parental animals in all groups (both generations) were euthanized by severing the brachial blood vessels following anesthesia with methoxyflurane, and subjected to a complete necropsy. The necropsy included examination of the external surfaces; all orifices; cranial cavity; carcass; external and cut surfaces of the brain and spinal cord; the thoracic, abdominal, and pelvic cavities and their viscera; and cervical tissues and organs. Of the 28 male and 28 female adults from the control and high exposure groups, ten/sex/group were selected randomly and subjected to a histopathology examination. Histopathologic evaluation was conducted on the tissues specified below after fixation in buffered neutral 10% formalin, paraffin embedment, and sectioning and staining with hematoxylin and eosin: pituitary, liver, kidneys (two), upper and lower respiratory tract (including nasal turbinates), vagina, uterus, ovaries, testes, epididymides, seminal vesicles, prostate, and other tissues with gross lesions identified as being potentially treatment related. Any of the above organs or tissues showing gross or histopathologic alterations, specifically the upper respiratory tract (including the nose, nasal turbinates, larynx, and trachea), were also evaluated microscopically in ten animals/sex from the other treatment groups.

A complete necropsy and histopathologic examination were conducted for any parental animals dying on test. The fixed (buffered neutral 10% formalin) uteri from any parental female of the F0 or F1 generations failing to produce a litter were stained with potassium ferricyanide for confirmation of pregnancy status; implantation sites (if any) were recorded. This procedure did not affect the subsequent histopathologic examination.

A gross internal examination was also performed on any pup appearing abnormal or dying on test, and on ten pups randomly selected for each sex from each test group of the F1 and F2 generations. As the necropsy of the randomly selected animals revealed no gross abnormalities, all remaining pups of all groups not used for the new parental generation were euthanized and discarded.

Statistical analyses. The unit of comparison was the male, the female, or the litter (Weil, 1970). Results of the quantitative continuous variables (e.g., body weights, organ weights, etc.) were intercompared for the three treatment groups and one control group by use of Levene's test for equal variances (Levene, 1960), analysis of variance (ANOVA), and *t*-tests. When Levene's test indicated homogeneous variances and the ANOVA was significant, the pooled *t*-test was used for pairwise comparisons. When Levene's test indicated heterogeneous variances, all groups were compared by an ANOVA for unequal variances (Brown and Forsythe, 1974) followed, when necessary, by the separate variance *t*-test for pairwise comparisons. The significance levels for the *t*-test comparisons were corrected by the Bonferroni method for all reproductive data.

Nonparametric data were statistically evaluated using the Kruskal-Wallis test (Sokal and Rohlf, 1969), followed by the Mann-Whitney *U* test for pairwise comparisons (Sokal and Rohlf, 1969), when appropriate. Frequency data (such as the various indices) were compared using the Fischer's exact test (Sokal and Rohlf, 1969). For all statistical tests, the fiducial limit of 0.05 (two-tailed) was used as the criterion for statistical significance.

This study was performed in 1987–1989 in compliance with U.S. Environmental Protection Agency (EPA) TSCA (Toxic Substances Control Act) good laboratory practice regulations (U.S. EPA, 1983) and in conformance with the U.S. EPA TSCA (U.S. EPA, 1985) and OECD (OECD, 1983) testing guidelines.

RESULTS

Chamber Analyses

The periodic analyses of the chamber atmospheres indicated that the mean analytical values of TDI ± SD for the low (0.02 ppm), middle (0.08 ppm), and high (0.30 ppm) exposure concentrations were 0.020 ± 0.003, 0.079 ± 0.009, and 0.29 ± 0.023 ppm, respectively. No test chemical was detected in the control atmospheres, with a minimum detection limit of 0.002 ppm (Table 1).

Percentage of the concentrations of the 2,4- and 2,6-isomers in the chamber atmospheres (sampled on exposure day 143) were 63.4% and 36.6% for the low concentration exposure chamber, 69.2% and 30.8% for the mid concentration exposure chamber, and 75.0% and 25.0% for the high concentration exposure chamber. These data indicated that the 2,4-isomer content of 80% decreased approximately 15%, 10%, and 5% in the low, mid, and high concentration exposure chambers, respectively. Although the test material stability was verified before and after the study, the isomer ratio of TDI in the exposure chamber did not precisely match the 80/20 ratio of the test material. This may relate to the imperfect match of analytical to nominal concentrations, suggesting some loss of the test material during the vapor generation process. Given the very low (sub ppm) range of test concentrations of this reactive material, a modest difference in analytical to nominal ratio and the isomer ratio was not surprising. The differences were unlikely to have had a significant impact on the outcome or interpretation of the study.

TABLE 1
Chamber Analyses

	Target concentrations (ppm)			
	0.00	0.02	0.08	0.3
Analytical concentrations (ppm)[a]				
Paper tape method	<MDL[b]	0.020 ± 0.0026	0.079 ± 0.0088	0.29 ± 0.023
Corrected by modified Marcali method	<MDL	0.018 ± 0.0023	0.070 ± 0.0077	0.23 ± 0.020
A/T ratio[c]				
Paper tape method	—	1.00	0.99	0.97
Corrected by modified Marcali method	—	0.90	0.88	0.77
Nominal Concentrations (ppm)[d]	—	0.045 ± 0.0049	0.127 ± 0.017	0.39 ± 0.027
A/N ratio[e]				
Paper tape method	—	0.44	0.64	0.74
Corrected by modified Marcali method	—	0.38	0.56	0.60
2,4-Isomer/2,6-isomer ratio[f]	—	1.7:1.0	2.2:1.0	3.0:1.0
Temperature (°C)[g]	24.1 ± 1.17	24.7 ± 0.99	24.2 ± 0.92	25.0 ± 1.02
Relative humidity (%)[g]	49.5 ± 4.80	46.0 ± 4.18	47.6 ± 4.49	47.6 ± 5.33

[a] Grand mean of 230 (0.02 ppm) or 235 (0.08 and 0.3 ppm) daily means ± standard deviation.
[b] Less than the **m**inimum **d**etection **l**imit of 0.002 ppm.
[c] Analytical to target concentration ratio.
[d] Grand mean of 229 (0.02 ppm), 234 (0.08 ppm), or 235 (0.3 ppm) daily means ± standard deviation.
[e] Analytical to nominal concentration ratio.
[f] Isomer ratio of the test chemical is 4.0:1.0 (80%/20%); these data are from "grab" samples taken during the exposures (see text).
[g] Grand mean of 233 (0.0 ppm), 230 (0.02 ppm) or 234 (0.08 and 0.3 ppm) daily means for temperature and relative humidity ± standard deviation.

Prebreed Exposures (F0)

During the 10-week prebreed exposure and 3-week mating periods of the F0 animals, male body weights were equivalent across all treatment groups (Fig. 1). Final male body weights (week 14) were statistically increased at 0.3 ppm. Male F0 weight gains at 0.3 ppm were reduced for the first exposure week and were significantly increased for treatment weeks 4–5 and 8–9; terminal F0 male body weight gains were significantly increased at 0.02, 0.08, and 0.3 ppm. Female F0 body weights (Fig. 2) exhibited no significant differences among groups during the prebreed exposure period or during the final exposure week. F0 female weight gains exhibited a similar equivalence across treatment groups for the prebreed exposure period. During the final week of exposure (week 18–19), females at 0.3 ppm exhibited a significantly increased weight gain. Treatment-related clinical signs of toxicity in F0 males included an increased incidence of nasal discharge at 0.3 ppm. F0 females exhibited an increased incidence of red-tinged fur about the head at 0.3 ppm. Periocular encrustation, perinasal encrustation, and/or red nasal discharge were observed in all exposure groups of F0 males and females, including the control group, and appeared to be associated with the inhalation treatment conditions *per se* rather than exposure to the test chemical vapor.

Gestation and Lactation (F0/F1)

Maternal F0 gestational and lactational body weights and weight gain (Fig. 2) were equivalent across all exposure groups. Reproductive parameters of F0 parents to produce F1 offspring were unaffected by treatment (Table 2). Gestational length was unaffected by exposure to the test chemical. F1 live birth and survival indices were unaffected by treatment (Table 2). F1 litter sizes, sex ratio (% males), and pup body weights and weight gains (by litter and by sex by litter) were equivalent across all treatment groups from lactational day 1 through 21 (Table 3). No treatment-related lesions were observed in the F1 pups, which died during the lactation period.

Necropsy and Histopathology (F0/F1)

Two F0 adult animals died during the conduct of this study: one female and one male. One F0 female at 0.02 ppm died of an abnormal pregnancy with uterine bleeding. The cause of death of the one F0 male at 0.3 ppm, found dead on study day 85, was not determined, although the animal had microscopic lesions of the respiratory tract similar to those of other animals in its exposure group.

After delivery of the F1 litters, all surviving F0 males were necropsied; after weaning of the F1 litters, all surviving F0 females were necropsied. Histopathology was performed on

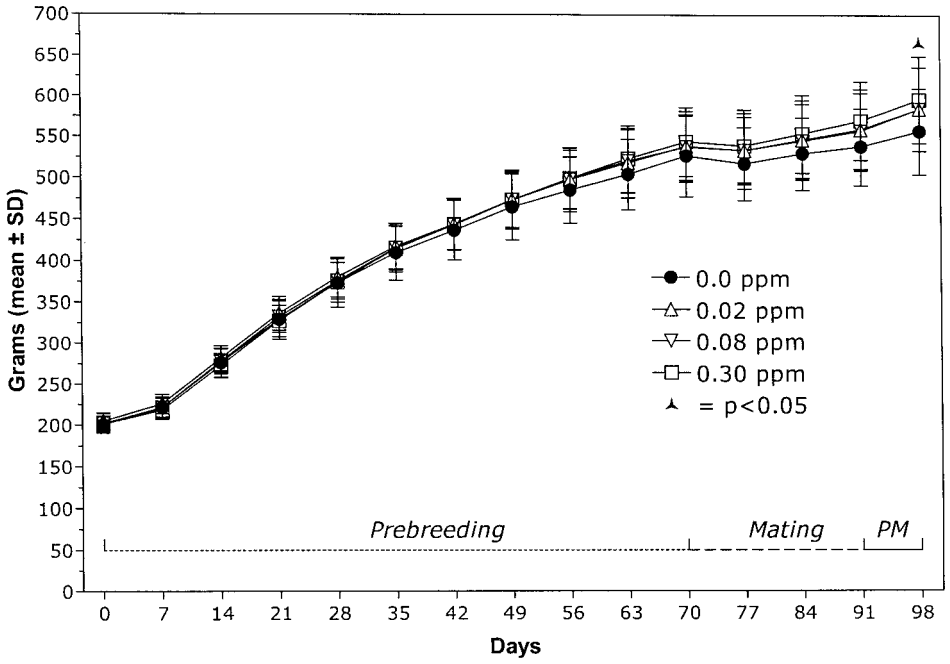

FIG. 1. Parental body weights (g ± SD), F0 males. PM, postmating exposure.

selected organs (pituitary, liver, kidneys, and reproductive organs) from ten parental animals per sex from high exposure concentration and control group animals. Histologic evaluation of nasal turbinates, larynx, and trachea from ten parental animals/sex was also performed for all exposure groups. There were no treatment-related gross lesions observed at necropsy. Treatment-related histopathologic lesions were limited to the upper respiratory tract, with tissues located deeper in the respiratory tract being less affected. In both F0 males and females at 0.3 ppm, the most frequently observed lesions were rhinitis and alterations (dysplasia and hyperplasia) of the respiratory (nasal) epithelium in the nasal turbinates. Increased incidences of rhinitis were also observed in nasal turbinates of F0 males and females at 0.08 and 0.02 ppm, relative to the F0 control males (one) and females (none). At 0.02 ppm, three F0 males exhibited rhinitis, one minimal multifocal and two mild multifocal; three F0 females at 0.02 ppm also exhibited rhinitis, one minimal multifocal and two mild (one each focal and multifocal) (Table 4). Necropsy of ten randomly selected F1 pups/sex/group indicated no treatment-related gross findings.

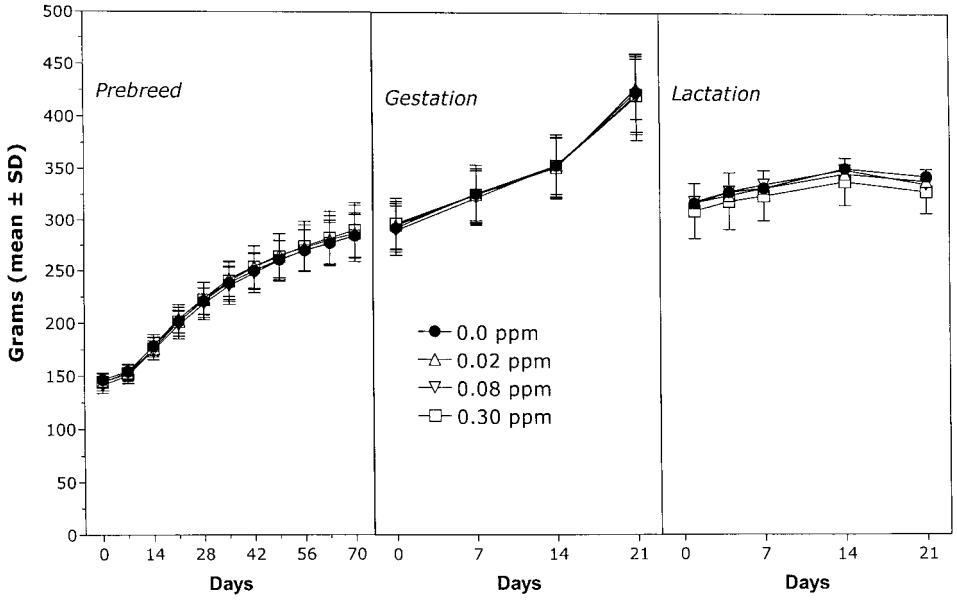

FIG. 2. Parental body weights (g ± SD), F0 females.

TABLE 2
Reproductive Parameters

	F0 (TDI, ppm)				F1 (TDI, ppm)			
	0.0	0.02	0.08	0.30	0.0	0.02	0.08	0.30
No. mating pairs	28	28	28	28	28	28	28	28
Mating index[a]	96.4	100.0	96.4	100.0	89.3	100.0	92.9	100.0
Fecundity index[a]	88.9	78.6	96.3	89.3	88.0	75.0	80.8	89.3
Fertility index[a,b]	85.7	78.6	92.9	89.3	78.6	75.0	75.0	89.3
Gestational index[a]	100.0	95.5	100.0	100.0	100.0	100.0	100.0	100.0
Gestational length, days	21.9 ± 0.3[c]	21.9 ± 0.4	21.9 ± 0.3	22.0 ± 0.06	21.9 ± 0.3	22.0 ± 0.6	22.0 ± 0.4	22.2 ± 0.5
No. live litters, pnd 0	24	21	26	25	22	21	21	25
No. live litters, pnd 21	23	20	25	24	21	21	20	25
Live birth index	97.7 ± 6.31[c]	97.0 ± 5.96	98.2 ± 4.71	98.7 ± 3.41	97.4 ± 4.67	95.2 ± 10.21	96.8 ± 7.76	98.5 ± 4.31
Survival indices[a]								
4-day (pnd 0–4 precull)	94.6 ± 20.35[c]	93.8 ± 21.71	93.9 ± 19.80	94.5 ± 20.12	94.3 ± 21.30	97.3 ± 3.95	93.9 ± 21.70	97.3 ± 6.16
7-day (pnd 4 postcull–7)	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.00 ± 0.00
14-day (pnd 7–14)	100.0 ± 0.00	100.0 ± 0.00	99.5 ± 2.50	99.5 ± 2.55	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
21-day (pnd 14–21)	100.0 ± 0.00	99.4 ± 2.80	100.0 ± 0.00	99.5 ± 2.55	99.4 ± 2.73	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00
Lactation index (pnd 4–21 postcull)	100.0 ± 0.00	99.4 ± 2.80	99.5 ± 2.50	99.0 ± 3.53	99.4 ± 2.73	100.0 ± 0.00	100.0 ± 0.00	100.0 ± 0.00

[a] Indices

$$\text{Mating index (\%)} = \frac{\text{Number of females with copulation plugs}}{\text{Number of females cohabited}} \times 100$$

$$\text{Fecundity index (\%)} = \frac{\text{Number of pregnancies}}{\text{Number of plug-positive females}} \times 100$$

$$\text{Fertility index (female) (\%)} = \frac{\text{Number of females pregnant}}{\text{Number of females cohabited}} \times 100$$

$$\text{Fertility index (male) (\%)} = \frac{\text{Number of males shown to be fertile}}{\text{Total number of males mated}} \times 100$$

$$\text{Gestational index (\%)} = \frac{\text{Number of females with live litters}}{\text{Number of females pregnant}} \times 100$$

$$\text{Live birth index (\%)} = \frac{\text{Number of live pups at birth}}{\text{Total number of pups born}} \times 100$$

$$\text{Survival indices (\%)} = \frac{\text{Number of live pups indicated on postnatal day}}{\text{Total number of live pups on previous index day}} \times 100;$$ from 7-day through lactation index, based on postcull survivors.

$$\text{Lactation index (\%)} = \frac{\text{Number of pups surviving 21 days}}{\text{Total number of live pups at 4 days (postcull)}} \times 100$$

[b] Fertility index is the same for both males and females; mating was 1:1 with no change in partners.
[c] Data are presented as mean ± SD.

Prebreed Exposures (F1)

During the 12-week prebreed exposure of the F1 animals randomly selected to be parents of the F2 generation, the males at 0.3 ppm exhibited reduced body weights (Fig. 3) relative to the controls for the first 4 weeks of exposure. For weekly intervals 0–1 and 1–2, as well as the final week of exposure (week 15–16), body weight gains were significantly reduced at 0.3 ppm. F1 males at 0.02 ppm exhibited significantly increased body weight gain relative to controls for weekly intervals 12–13 and 13–14 of the mating period. The F1 females exhibited reduced body weights at 0.3 ppm for the first 2 weeks of exposure, as well as week 6 of exposure (Fig. 4). There were no significant differences among groups for F1 female weight gain. In F1 males, there were no significant treatment- or concentration-related changes in incidence of clinical observations. Although perinasal encrustation was observed across all treatment groups in F1 females, the incidence was significantly increased at 0.3 ppm. The incidence of red-tinged fur, although occasional in TDI-exposed F1 males, was significantly increased in F1 females at 0.08 and 0.3 ppm from 17 to 22 weeks (study days 120–155) of exposure.

Gestation and Lactation (F1/F2)

Maternal F1 gestational and lactational body weight and body weight changes were unaffected for all time points measured (Fig. 4). Gestational length was unaffected by exposure. F2 pup live birth and survival indices were unaffected by treatment (Table 2). F2 litter size and sex ratio (% males) were unaffected by treatment (Table 3). At 0.3 ppm, F2 pup body weight per litter exhibited reductions (males, females, and all

TABLE 3
Offspring Litter Size, Sex Ratio, Body Weights, and Weight Gain during Lactation

	F1 offspring (TDI, ppm)				F2 offspring (TDI, ppm)			
	0.0	0.02	0.08	0.30	0.0	0.02	0.08	0.30
pnd 0								
No. live litters	24	21	26	25	22	21	21	25
No. total pups/litter	13.8 ± 2.51[a]	14.1 ± 2.14	13.1 ± 3.19	14.2 ± 3.53	13.9 ± 3.35	12.8 ± 3.36	13.6 ± 2.91	13.6 ± 2.66
No. live pups/litter	13.5 ± 2.30	13.6 ± 1.94	13.0 ± 3.34	14.0 ± 3.49	13.5 ± 3.38	12.4 ± 3.65	13.1 ± 3.05	13.4 ± 2.74
% males/litter	49.6 ± 12.9	48.0 ± 12.4	45.6 ± 14.5	50.7 ± 15.7	48.6 ± 13.3	50.8 ± 15.3	48.6 ± 20.2	46.5 ± 12.5
pnd 1								
No. live litters	23	21	26	24	22	21	21	25
Pup body weight/litter[b]	7.20 ± 0.72	7.06 ± 0.78	7.04 ± 0.75	6.73 ± 0.71	7.08 ± 1.06	7.09 ± 1.03	7.04 ± 0.70	7.10 ± 0.75
pnd 4 (precull)								
No. live litters	23	20	25	24	21	21	20	25
No. pups/litter	13.3 ± 2.40	13.5 ± 1.96	13.0 ± 2.94	14.3 ± 2.18[c]	13.2 ± 3.35	12.1 ± 3.65	13.2 ± 2.86	13.0 ± 2.74
% males/litter	50.3 ± 13.0	47.8 ± 12.3	45.5 ± 14.6	53.0 ± 11.9	48.8 ± 13.7	51.4 ± 15.8	50.1 ± 20.0	46.1 ± 12.5
Pup body weight/litter	10.19 ± 1.00	9.71 ± 1.12	9.96 ± 1.37	9.46 ± 1.27	10.19 ± 1.82	10.48 ± 1.88	9.93 ± 1.13	10.28 ± 1.28
Pup weight gain/litter, pnd 1–4[b]	2.99 ± 0.73	2.60 ± 0.80	2.92 ± 0.74	2.72 ± 0.70	3.09 ± 1.02	3.39 ± 1.07	2.89 ± 0.83	3.18 ± 0.74
pnd 7								
No. live litters	23	20	25	24	21	21	20	25
No. pups/litter[d]	8.0 ± 0.00	8.0 ± 0.00	7.9 ± 0.28	8.0 ± 0.00	7.8 ± 1.09	7.5 ± 1.50	7.8 ± 1.12	8.0 ± 0.20
% males/litter	50.0 ± 6.5	48.8 ± 3.8	46.2 ± 9.5	50.5 ± 4.5	50.8 ± 5.4	50.4 ± 7.7	51.9 ± 14.2	50.3 ± 3.9
Pup body weight/litter	14.89 ± 1.25	14.42 ± 1.56	14.91 ± 1.59	14.02 ± 1.66	15.38 ± 2.41	15.25 ± 2.13	14.23 ± 1.66	14.68 ± 1.46
Pup weight gain/litter, pnd 4–7	4.70 ± 0.60	4.71 ± 0.72	4.95 ± 0.66	4.57 ± 0.78	5.19 ± 0.92	4.77 ± 0.72	4.29 ± 1.08**	4.39 ± 0.93*
pnd 14								
No. live litters	23	20	25	24	21	21	20	25
No. pups/litter	8.0 ± 0.00	8.0 ± 0.00	7.9 ± 0.33	8.0 ± 0.20	7.8 ± 1.09	7.5 ± 1.50	7.8 ± 1.12	8.0 ± 0.20
% males/litter	50.0 ± 0.0	48.8 ± 3.8	45.9 ± 9.5	50.7 ± 3.9	50.8 ± 5.4	50.4 ± 7.7	51.9 ± 14.2	50.3 ± 3.9
Pup body weight/litter	28.14 ± 2.17	27.74 ± 3.21	28.72 ± 2.42	26.71 ± 2.67	29.79 ± 3.56	29.35 ± 3.50	27.51 ± 2.24*	27.33 ± 2.13*
Pup weight gain/litter, pnd 7–14	13.25 ± 1.92	13.32 ± 2.10	13.81 ± 1.53	12.69 ± 1.80	14.41 ± 1.64	14.10 ± 1.94	13.28 ± 1.25	12.66 ± 1.42**
pnd 21								
No. live litters	23	20	25	24	21	21	20	25
No. pups/litter	8.0 ± 0.00	8.0 ± 0.00	7.9 ± 0.33	7.9 ± 0.28	7.7 ± 1.10	7.5 ± 1.50	7.8 ± 1.12	8.0 ± 0.20
% males/litter	50.0 ± 6.5	48.4 ± 4.0	45.9 ± 9.5	51.0 ± 4.0	50.5 ± 5.69	50.4 ± 7.7	51.9 ± 14.2	50.3 ± 3.9
Pup body weight/litter	46.17 ± 3.87	45.74 ± 4.59	46.99 ± 3.86	44.51 ± 4.88	49.03 ± 5.79	48.97 ± 6.20	46.18 ± 3.68	45.47 ± 3.64[e]
Pup weight gain/litter, pnd 14–21	18.04 ± 2.19	18.00 ± 1.92	18.27 ± 2.10	17.76 ± 2.66	19.23 ± 2.88	19.62 ± 2.99	18.67 ± 1.83	18.14 ± 1.81

[a] Data are presented as mean ± S.D.
[b] Pup body weight and weight gain in grams, sexes combined.
[c] The mean number of pups/litter was higher on pnd 4 than on pnd 0, since one litter with seven live pups, present on pnd 0 (so $n = 25$) had no live pups on pnd 4 (so n = 24).
[d] Litters culled to eight pups on pnd 4.
[e] Pup body weight per litter was significantly reduced ($p < 0.05$) for male pups only, not for female or total pups per litter.
* $p < 0.05$ versus control group value.
** $p < 0.01$ versus control group value.

pups) beginning on pnd 14 and persisting through day 21 for male pups. Body weights of female pups and all pups/litter were also reduced on lactation day 14 at 0.08 ppm. Pup body weight gains per litter (males, females, and all pups) were reduced at 0.08 and 0.3 ppm for pnd 4 to 7, and at 0.3 ppm pup body weight gain reductions persisted (males, females, and all pups) through pnd 14 (Table 3).

Necropsy and Histopathology (F1/F2)

Three F1 females were sacrificed prior to scheduled sacrifice and included one animal at 0.08 ppm on study day 94 due to traumatic injury, and one animal each at 0.3 ppm and 0.08 ppm due to early deliveries during exposure.

After delivery (F1 males) or weaning (F1 females) of the F2 litters, all surviving F1 parental animals were necropsied. No gross treatment-related lesions were observed. Selected tissues were examined histologically on ten animals per sex from high exposure and control animals. The one target tissue, the upper respiratory tract, including nasal turbinates, larynx, and trachea, was also examined microscopically from ten animals/sex from all groups. As with F0 parents, histologic lesions were limited to the upper respiratory tract (nasal cavities, larynx, and

TABLE 4
Incidence of Rhinitis in F0 and F1 Parental Animals

	F0 (TDI, ppm)				F1 (TDI, ppm)			
	0.0	0.02	0.08	0.30	0.0	0.02	0.08	0.30
Males								
No. examined	10	10	10	10	10	10	10	10
Rhinitis	1	3	8	9	0	7	5	9
Minimal	0	1	0	0		6	3	2
Focal		0				0	0	1
Multifocal		1				6	3	1
Diffuse		0				0	0	0
Mild	1	2	4	6		0	1	3
Focal	0	0	0	0			0	0
Multifocal	1	2	4	3			1	3
Diffuse	0	0	0	3			0	0
Moderate	0	0	4	3		1	1	4
Focal			0	0		0	0	0
Multifocal			4	2		1	1	4
Diffuse			0	1		0	0	0
Females								
No. examined	10	10	10	10	10	10	10	10
Rhinitis	0	3	6	9	0	4	5	9
Minimal		1	2	2		1	4	5
Focal		0	0	0		1	0	0
Multifocal		1	2	2		0	4	5
Diffuse		0	0	0		0	0	0
Mild		2	3	4		1	1	3
Focal		1	0	0		0	0	0
Multifocal		1	2	4		1	1	3
Diffuse		0	1	0		0	0	0
Moderate		0	1	3		2	0	1
Focal			0	0		0		0
Multifocal			0	3		2		0
Diffuse			1	0		0		1

Note. The upper respiratory tracts, including the nose, nasal turbinates, larynx, and trachea, from ten parental animals/sex/group/generation, were examined histopathologically. The findings for rhinitis are presented by incidence, degree (minimal, mild, or moderate), and distribution (focal, multifocal, or diffuse).

trachea). Although dysplasia and/or hyperplasia of the nasal respiratory epithelium were present in F1 parents at 0.08 and 0.3 ppm, the frequency of occurrence was not significantly increased relative to the control frequency. Rhinitis was observed with increased frequencies in all TDI-exposed groups of F1 males and females (with no rhinitis observed in the control animals). Seven of the ten F1 males and four of the ten F1 females examined at 0.02 ppm exhibited rhinitis; in the seven males with rhinitis, six were classified as minimal multifocal and one as moderate multifocal; in the four females with rhinitis, one was minimal focal, one was mild multifocal, and two were moderate multifocal (Table 4). Mononuclear cell infiltration of liver tissue, although present in controls and significantly increased in F1 females at 0.3 ppm, was not deemed treatment related. No treatment-related gross lesions were observed in F2 pups that died during lactation, or in the ten/sex/group subjected to necropsy at weaning.

DISCUSSION

The present study, evaluating exposure of CD® (Sprague-Dawley) rats to TDI by inhalation for two generations, one litter per generation, has shown a moderate pattern of adult toxicity at 0.3 ppm, evidenced by occasionally reduced body weights in F0 males and F1 males and females and/or weight gains during the prebreed exposure period (more frequently in the F1 animals). Body weight gains were sporadically increased for F0 males and females at 0.3 ppm during the prebreed (males) and postwean (females) exposure periods, in F0 males following the mating period at 0.02, 0.08, and 0.3 ppm, and for F1 males during mating at 0.02 ppm (but not at 0.08 or 0.3 ppm). The significance, if any, and the explanation for the increased body weights and weight gains in F0 males and females and in F1 males, are not known; these findings are probably not toxicologically significant.

Clinical signs of toxicity attributable to treatment at 0.3 ppm

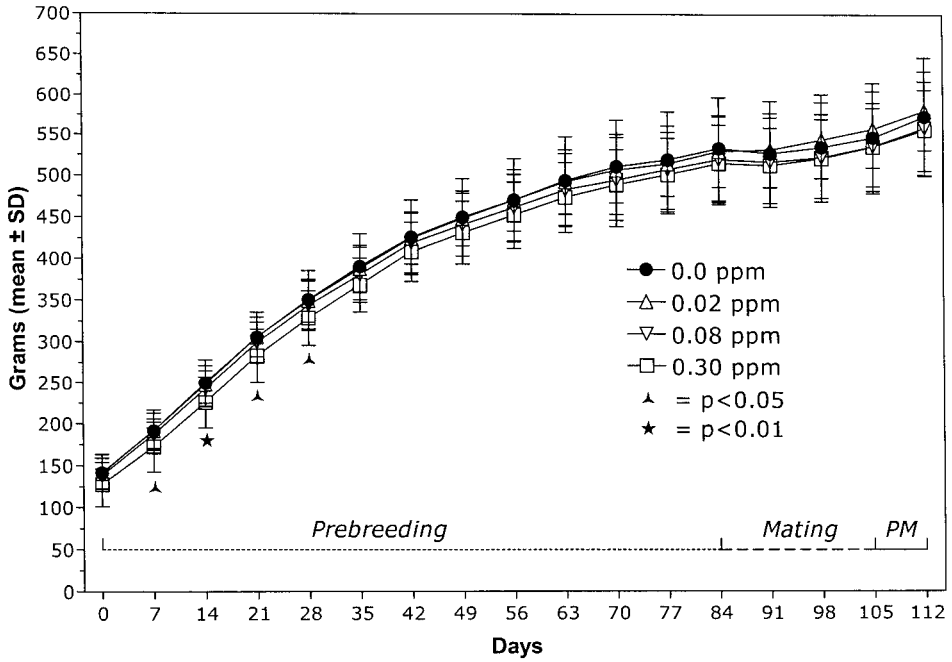

FIG. 3. Parental body weights (g ± SD), F1 males. PM, postmating exposure.

included significantly increased incidences of nasal discharge in F0 males and of red-tinged fur in the head region in F0 females. F1 females exhibited significantly increased incidences of perinasal encrustation at 0.3 ppm and of red-tinged fur at 0.08 and 0.3 ppm. The red-tinged fur is from chromodacryorrhea, red-brown material (porphyrin) secreted from the Harderian gland and distributed about the face and neck by normal grooming activity (Seely, 1987). It is a relatively nonspecific indication of stress. It is likely that this stress-related finding is associated with the longer duration of females' exposure to TDI. (F0 and F1 males were sacrificed on days 95–96 and 108–109 of exposure, respectively, prior to the time when the finding was observed in the F0 females at days 124–132 and F1 females at days 120–155.)

Significant treatment-related histologic findings were limited to changes in the upper respiratory tract, including minimal to moderate rhinitis at all exposure levels in F0 and F1 adult males and females. At 0.02 ppm, there were three (of ten) F0 males, seven F1 males, three F0 females, and four F1 females; at 0.08 ppm, there were eight (of ten) F0 males, five

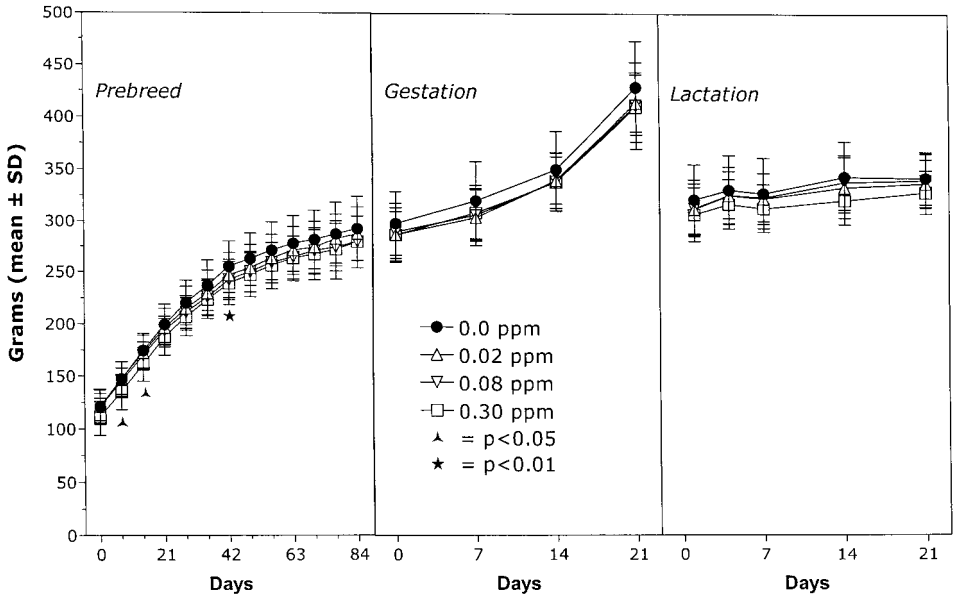

FIG. 4. Parental body weights (g ± SD), F1 females.

F1 males, six F0 females, and four F1 females; at 0.30 ppm, there were nine (of ten) F0 males, nine F1 males, nine F0 females, and nine F1 females, relative to the control incidence of no F1 males and F0 and F1 females, and one F0 male, out of ten/sex/group examined in each generation. Rhinitis is a typical response of the rodent to an irritant material, and similar effects have been reported in response to well-known irritants such as chlorine (Barrow *et al*., 1979), acrolein (Feron *et al*., 1978), sulfur dioxide (Giddens and Fairchild, 1972), and acetaldehyde (Kruysse *et al*., 1975). The rat is regarded as the most sensitive of the commonly used laboratory species in its response to these irritants. This is exemplified by acrolein, where Feron *et al*. (1978) showed that the rat, rabbit, and hamster all showed effects at 4 ppm, but rhinitis accompanied by hyperplasia and metaplasia occurred in the rat at 0.4 ppm acrolein, which was a no-effect level in the other species. These and similar results are taken as an indication that a mild irritant response in the rat should not be regarded as being of major toxicologic significance. Because the rhinitis is localized in the most anterior portion of the upper respiratory tract, the proximate site of contact of TDI, this finding can be viewed as an essentially localized, nonspecific response to exposure to an irritating vapor. Other histologic findings only at 0.3 ppm, specifically dysplasia and/or hyperplasia of the nasal respiratory epithelium, were indicative of more severe irritant effects of TDI. These effects of TDI have similarities to those found after long-term exposure to MDI aerosol in rats (e.g., Heinrich *et al*., 1991; Hoymann *et al*., 1994; Klingebiel *et al*., 1991; Martin-Carrera *et al*., 1991; Reuzel *et al*., 1994a,b). The increased incidence of mononuclear cell infiltration in the livers of F1 females at 0.30 ppm was not deemed treatment related by the study pathologist due to its common background occurrence in rats, its presence in all groups, including the control in both generations, and no evidence of increased incidence in F1 males or in F0 males and females at 0.30 ppm.

No reproductive parameters were affected during either generation (F1 or F2). During the breeding phases for the F0 and F1 females, there were no treatment-related changes in gestational body weights or lactational body weights. F2 (but not F1) litters exhibited reduced body weights at 0.3 ppm for lactational days 14 and 21, and at 0.08 ppm for lactational day 14 only. Lactational body weight gains were reduced in F2 (but not F1) litters at 0.08 ppm for lactational days 4–7 and at 0.3 ppm for lactational days 4–7 and 7–14. Reduced pup body weight gain per litter at 0.08 and 0.3 ppm, observed during the lactation period (only for the F2 generation), initially occurred during the interval lactational days 4–7, when removal of the dams from the nest was reinstated for exposures (beginning on lactational day 5) in all groups. Removal of the dams for more than 6 h/day reduced pups′ access to their food supply (the lactating dam) and was most likely compounded by the dams' discomfort at 0.08 and 0.3 ppm upon return to the nest after the daily exposures. The effects at 0.08 ppm were resolved during the last week of lactation. There were no effects on F1 or F2 offspring body weights or weight gains at 0.02 ppm.

Continued inhalation exposure to TDI vapor for two generations in CD® (Sprague-Dawley) rats resulted in parental toxicity at 0.02, 0.08, and 0.3 ppm, evidenced by occasional body weight and weight gain depression and clinical signs of toxicity at 0.08 and 0.3 ppm, and histologic changes in the nasal cavities in both sexes and both generations at 0.02, 0.08, and 0.3 ppm. Postnatal toxicity, consisting of reduced body weights and body weight gains, occurred only in F2 litters at 0.08 and 0.3 ppm. There was no effect of treatment on reproductive organs or functions. No adult no observable adverse effect level (NOAEL) was identified, although the rhinitis observed at 0.02 ppm was considered a mild, nonspecific response to an irritant. The reproductive NOAEL was at least 0.3 ppm, and the postnatal toxicity NOAEL was 0.02 ppm in rats, under the conditions of this study.

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