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February 23, 1990

Janine Mifsud
Information Scientist
Syracuse Research Corp.
Merrill Lane
Syracuse, NY 13210

Dear Ms. Mifsud,

You recently requested a copy of the report on the Inhalation Developmental Toxicity Study of 1,1,1-Trichloroethane which is available from the Washington, DC office of the Halogenated Solvents Industry Alliance. During this conversation, we also discussed other developmental toxicity studies that have been conducted on this material in the last few years, and you requested the key references in this area.

Several past studies had shown no significant developmental toxicity effects due to this chemical. In 1984, Dapson et al. (Teratology 29 25A, 1984) reported cardiac malformations in 21-day old rats born to dams exposed through the drinking water. Subsequently, the National Toxicology Program and the National Institute of Environmental Health Sciences contracted to conduct a study to determine the repeatability of the effects reported in the Dapson study. This study has been completed and the results of this work showed no evidence for reproductive toxicity or teratogenic effects produced by 1,1,1-trichloroethane.

The results from the NTP/NIEHS study were first reported in 1987 at the 26th Annual Meeting of the Society of Toxicology (Toxicologist 7 (1), 175, 1987). A copy of the abstract of the presentation is enclosed. This work has been published recently by J. D. George et al. (Fundamental and Applied Toxicology 13 641-651, 1989). A copy of the paper is enclosed. The complete work is available as two reports:

> George, J.D., Price, C.J., Marr, M.C., Morrissey, R.E., and Schwetz, B.A. Developmental Toxicity Evaluation of 1,1,1-Trichloroethane (CAS No. 71-55-6) Administered to CD Rats.

Part I. Postnatal Evaluation, Final Study Report. NTP/NIEHS Contract N01-ES-55080, Aug. 28, 1987. NTIS Accession No. PB88131321/AS.

George, J.D., Price, C.J., Marr, M.C., Morrissey, R.E., and Schwetz, B.A. Developmental Toxicity Evaluation of 1,1,1-Trichloroethane (CAS No. 71-55-6) Administered to CD Rats. Part II. Teratological Evaluation, Final Study Report. NTP/NIEHS Contract N01-ES-55080, Sept. 23, 1987. NTIS Accession No. PB88134101.

As you can see, the results of the NTP/NIEHS study confirm the conclusions of the earlier studies, and the findings of the Dapson study, which are inconsistent with the earlier studies, were not verified by the NTP/NIEHS study.

Please feel free to contact me if you want to discuss this issue further.

Yours truly,

James A Barter

James A. Barter, Ph.D., D.A.B.T.

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38 EFFECTS OF AFLATOXINS B1 AND G1 ON POSTIMPLANTATION RAT EMBRYOS. E.A. Maull, B.A. Clement, N.D. Heidelbaugh and T.D. Phillips. Veterinary Public Health, Texas A&M University, College Station, TX.

In vitro studies were performed to determine the potential for interactions between Aflatoxin B1 (AFB1) and Aflatoxin G1 (AFG1) in postimplantation embryos. Embryos were collected on day 10.5 of gestation, dissected from uterus and decidua and rolled continuously for 45 hours in gassed rat serum. Viability was assessed by yolk sac diameter, crown-rump length and somite number. Optimal concentrations of AFB1 (5 μM) and AFG1 (10 μM) were tested with and without an S9 hepatic metabolizing system. The combined effects of AFB1 and AFG1 resulted in an increase in yolk sac diameter when compared to the two toxins alone; while, the crown-rump length and somite number remained unchanged. Surprizingly, the addition of an S9 fraction to the treatment groups provided some protection from the toxic effects. This finding suggests that prior metabolism may result in nonspecific binding and/or inactivation under the experimental conditions employed. Thus, the parent compounds may or may not require activation in the yolk sac or embyro for toxicity. Further investigations (employing monoclonal antibodies) are ongoing to quantitatively assess yolk sac and embryonal dosimetry. (Supported by USDA Project 84CRSR-2-2434, TAES H6215 and AH 6830.)

700 DEVELOPMENTAL TOXICITY OF 1,1,1-TRICHLOROETHANE (TCEN) IN CD RATS. R.D. George, C.J. Price, M.C. Marr, B.A. Schwetz* and R.E. Morrissey*, Research Triangle Institue and *NTP/NIEHS. Research Triangle Park, NC

1,1,1-Trichloroethane (TCEN) is an industrial solvent that has been found in contaminated water supplies. Cardiac malformations have been reported in 21-day old rats born to dams exposed prenatally and throughout lactation to 10 ppm TCEN in the drinking water (Dapson et al., Teratology 29, 25A, 1984). In the present study, male and female CD rats (>20/group) were given deionized/filtered water, 0.05% Tween 80 with 0.9 ppm 1,4-dioxane (vehicle control), 3, 10, or 30 ppm TCEN for 2 weeks prior to mating and during mating. Sperm-positive females were treated at the same dose levels throughout gestation and lactation. TCEN blood levels were determined in the F_0 males and females after premating and mating, and in the F_0 females after lactation. Pups were evaluated for visceral malformations and for TCEN blood levels on postnatal day 21. There was no evidence of an increase in cardiac or other visceral malformations in the pups at any dose level of TCEN. These results are in contrast to the published preliminary findings noted above. Supported by NTP/NIEHS Contract No. NO1-ES-55080.

39 TERATOGENIC EFFECTS OF ORGANOPHOSPHORUS INSECTICIDES IN Xenopus laevis. J.E. Snawder and J.E. Chambers. Dept. of Biological Sciences, Mississippi State University, Mississippi State, MS

Since organophosphorous insecticides have been shown to be teratogenic in developing chicken embryos, they have been studied for their teratogenic potential in Xenopus laevis embryos. Eggs were exposed to monocrotophos, dicrotophos, malathion, malaoxon, parathion or paraoxon for 96 h. Concentrations of compounds ranged from 0.1 mg/l to 25 mg/l in water. At the end of 96 h the embryos were collected, measured, examined for morphological defects, and assayed for their concentrations of NAD. All the insecticides examined caused morphological defects and lowered NAD levels in a dose-dependent manner. The IC_{50} values in mg/l for each compound were: dicrotophos, 2.5; monocrotophos 10.0; malathion, 0.25; malaoxon, 0.25; parathion, 1.0; and paraoxon, 0.1. The LC_{50} values in mg/l were: malathion, 12.5; malaoxon, 1.0; parathion, 15.0; and paraoxon, 1.0. LC_{50} values for dicrotophos and monocrotophos are in excess of 10.0 mg/l. These results suggest that the organophosphorous insecticides do have teratogenic potential at concentrations that may be found in the environment, especially in temporary pools where many amphibian species reproduce.

701 THE EFFECT OF PHENYLETHYL ALCOHOL APPLIED DERMALLY TO PREGNANT RATS. R.A. Ford, A.M. Api, Research Institute for Fragrance Materials Inc., Englewood Cliffs, NJ and T.M. Palmer, Huntingdon Research Centre Ltd., Cambridgeshire, England. Sponsor: O.E. Easterday.

Phenylethyl alcohol (PEA) is an important and widely distributed fragrance ingredient due to having the characteristic odor of roses. It is also a natural constituent of roses. A study of the potential effects of dermally applied PEA on pregnant rats was conducted by applying undiluted PEA under 24 hr./day occlusion in doses of 0.14, 0.43 or 1.4 ml/kg daily during days 6 to 15 of pregnancy. The high dose resulted in maternal toxicity accompanied by a broad spectrum of fetal effects including morphological abnormalities in virtually all fetuses. The mid-dose did not produce maternal toxicity or fetal effects other than very small cervical rib buds in 30/129 fetuses. The low dose did not produce any definitive evidence of adverse effect. The 0.14 ml/kg dose is about 250 times higher than the calculated 90%ile exposure dose from the use of PEA in fragranced products. Comparative rat/human blood level concentrations increase this safety factor to approximately 10,000.

FUNDAMENTAL AND APPLIED TOXICOLOGY 13, 641–651 (1989)

Developmental Toxicity of 1,1,1-Trichloroethane in CD Rats[1]

JULIA D. GEORGE,*[,2] CATHERINE J. PRICE,* MELISSA C. MARR,* BRIAN M. SADLER,* BERNARD A. SCHWETZ,† LINDA S. BIRNBAUM,† AND RICHARD E. MORRISSEY†

*Chemistry and Life Sciences, Center for Life Sciences and Toxicology, Research Triangle Institute, P.O. Box 12194, Research Triangle Park, North Carolina 27709-2194, and †National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

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Developmental Toxicity of 1,1,1-Trichloroethane in CD Rats. GEORGE, J. D., PRICE, C. J., MARR, M. C., SADLER, B. M., SCHWETZ, B. A., BIRNBAUM, L. S., AND MORRISSEY, R. E. (1989). *Fundam. Appl. Toxicol.* **13**, 641–651. 1,1,1-Trichloroethane (TCEN), a major industrial and household solvent, was evaluated for pre- and postnatal developmental effects in Sprague–Dawley rats. This study was designed to assess the repeatability of a report (S. C. Dapson, D. E. Hutcheon, and D. Lehr, *Teratology* **29**, 25A, 1984) that indicated that 10 ppm TCEN in drinking water caused cardiac malformations in developing rats. In the present study, TCEN (97% pure) was administered in the drinking water at target concentrations of 3, 10, and 30 ppm, using 0.05% Tween 80 as an emulsifying agent. Two control groups, one receiving deionized/filtered water and the other receiving a vehicle control solution containing 0.05% Tween 80 and 0.9 ppm 1,4-dioxane, a stabilizing agent found in the bulk chemical, were also included. Male and female breeders (more than 30 per group) were exposed to the control solutions or test compound for 14 consecutive days prior to cohabitation and for up to 13 days during the cohabitation phase. Sperm-positive females (24–29 per group) continued to be exposed to these formulations during pregnancy and lactation to Postnatal Day (PND) 21. Parental animals exhibited a slight aversion to the 30-ppm drinking water during the premating exposure. No significant effect on reproductive competence of the parental animals or postnatal growth and development of the offspring to PND 21 was noted. A slight increase in mortality from implantation to PND 1, possibly due to high mortality in one litter, was observed in the 30-ppm dose group. There was no indication of an increase in the incidence of cardiac or other malformations in PND 21 pups. In summary, TCEN administered at 3, 10, and 30 ppm in the drinking water had no significant effect on the morphological development of CD rats.

1,1,1-Trichloroethane (TCEN) is a saturated lipophilic, trichlorinated hydrocarbon used primarily as an industrial solvent for cleaning metals (Stewart, 1968). It is also used in dry cleaning, in vapor degreasing, as an aerosol propellant, as a developing solvent for printed circuit boards, and as a solvent in household products (IARC, 1979). In 1985, the United States produced 869 million pounds of TCEN (C&E News, 1987). The current threshold limit value recommended for TCEN is 350 ppm (1900 mg/m^3), determined as a time-weighted average for an 8-hr exposure period (ACGIH, 1987).

Several laboratory investigations of TCEN have indicated no significant adverse effect on reproductive capacity. Pregnant Sprague–Dawley rats and Swiss Webster mice exposed by inhalation to 875 ppm commercial-grade TCEN (Chlorothene VG, 94.5% TCEN, 5.5% inhibitors and impurities) for 7 hr daily on

[1] Presented at the 26th Annual Meeting of the Society of Toxicology, Washington, DC, February 24–27, 1987. *Toxicologist* 7(1), 175, 1987.

[2] To whom all correspondence should be addressed.

0272-0590/89 $3.00

Gestation Days (GD) 6–15 (mean exposure 2404 mg/kg/day for rats or 3500 mg/kg/day for mice) exhibited no significant changes in maternal body weight, fetal viability, or morphological development at term, when compared with concurrent controls (Schwetz *et al.*, 1975). In a multigeneration reproduction study conducted with ICR Swiss mice, TCEN (97% pure, stabilized with 3% 1,4-dioxane) was administered in the drinking water (containing 1% Emulphor EL-620 emulsifier) at concentrations selected to provide nominal doses of 0, 583, 1749, and 5833 ppm [mean exposure 87–870 mg/kg/day (Lane *et al.*, 1982)]. A deionized/filtered water control group was also included. Exposure was continuous throughout the experiment. Exposure to TCEN produced no significant evidence of reproductive or developmental toxicity in the parental generation (F_0) or any offspring (i.e., the F_{1a}, F_{1b}, and F_{1c} litters, or the F_{2a} and F_{2b} litters produced from the F_{1b} litter).

In 1981, in Santa Clara County, California, a well was removed from service 3 weeks after contamination with TCEN and other solvents. At that time, the TCEN level in the water had reached 1700 ppb. This was followed by anecdotal reporting of increased incidences of spontaneous abortion and congenital malformations including cardiac defects. As a result, the California Department of Health Services (CDHS) conducted two epidemiological studies: (1) an examination of pregnancy outcome of residents in the contaminated area compared with residents in a demographically similar area having an uncontaminated water supply; and (2) an examination of the incidence of congenital cardiac defects in the contaminated area compared with incidence rates for the entirety of Santa Clara County (CDHS, 1982). Both studies focused on women who conceived during the period January 1, 1980, to December 31, 1981. No direct correlation could be drawn between exposure to the TCEN-contaminated water and the increased incidence of spontaneous abortion or congenital anomalies. Shortly thereafter, Dapson *et al.* (1984) reported an increase in cardiac anomalies in TCEN-exposed developing rats. In that study, male and female Sprague–Dawley rats received either drinking water containing 10 ppm TCEN (97% pure, stabilized with 3% 1,4-dioxane) dispersed with 0.05% Tween 80 or vehicle control water containing 0.05% Tween 80 for 7 days prior to cohabitation and up to 21 days after cohabitation and prior to conception (mean total exposure to TCEN for males and females prior to conception was 20.4 days). Sperm-positive females continued to receive treatment throughout the gestational and lactational periods. Offspring from five treated litters and six control litters were evaluated for growth and physical development on Postnatal Days (PND) 21–23. No significant differences were observed in mean body weights or in the mean wet heart weights of the two groups; however, cardiac abnormalities consisting of persistent ductus arteriosus and right and/or left atrial hypoplasia or displacement were observed in approximately 30% (15/52) of the TCEN-exposed offspring. This incidence was significantly higher than observed in the control offspring. The mean TCEN level in the serum from eleven 78-day-old pups from one TCEN-treated litter continuously exposed to 10 ppm TCEN was 0.03 μg/ml (30 ng/ml) (Dapson, personal communication).

Because of the importance of TCEN as an industrial and household chemical and the preliminary results of Dapson *et al.* (1984), which were in conflict with other laboratory studies (see above), additional studies were conducted in our laboratory using a study design similar to but more extensive than that of Dapson *et al.* and under experimental conditions more controlled than those used by Dapson *et al.* (1984). Offspring were evaluated at term (GD 20) or on PND 21 (George *et al.*, 1987a,b). In the teratology study, TCEN (99% pure) was administered in the drinking water at a target concentration of 3, 10, or 30 ppm, using 0.05% Tween 80 as an emulsifying agent. Two control groups, one

receiving deionized/filtered water and the other a vehicle control solution containing 0.05% Tween 80, were also included. Male and female breeders (more than 30 per group) were exposed to the control solutions or test compound for 14 consecutive days prior to cohabitation and for up to 6 days during cohabitation. Sperm-positive females (26–30 per group) continued to be exposed to these formulations through GD 20. TCEN had no significant effect on parental body weight, food consumption, or water consumption, compared with the vehicle control group. In addition, TCEN had no effect on prenatal viability or growth, and did not alter morphological development of fetuses observed on GD 20. Further details of this study can be found in George *et al.* (1987b). The postnatal evaluation of TCEN is described in the present article.

MATERIALS AND METHODS[3]

Animals and animal husbandry. Sprague–Dawley rats (Crl:CD BR VAF/Plus outbred albino rats)[4] were 8–12 weeks of age at the time of arrival, and were quarantined 1 week prior to study initiation. Rats were housed on Ab-Sorb-Dri cage litter[5] in solid-bottom polycarbonate cages (19 × 10½ × 8 in.) with stainless-steel wire lids[6] and molded filter tops.[7] Males were housed singly except during cohabitation; females were group housed (maximum of three per cage) during quarantine and singly housed after cohabitation. Food[8] and drinking water (control, vehicle control, or TCEN-containing) were available *ad libitum* throughout the study. Animal rooms were equipped with automatic light cycles (lights on from 7:00 AM to 7:00 PM). Relative humidity and temperature were maintained at 60 ± 6% and 71 ± 1°C, respectively.[9] Estimated exchange rate for air in each animal room was 12 to 14 times per hour.

Male and female rats were randomly distributed into three equal cohorts (A, B, or C), with exposure to the compound beginning by cohort on 3 successive days. Animals were assigned to dose groups in cohorts so that body weight on the first day of the premating exposure was not significantly different across dose groups within individual cohorts, or for the entire study.

Test chemical and treatment. 1,1,1-Trichloroethane (CAS No. 71-55-6) of 97% purity stabilized with 3% 1,4-dioxane[10,11] was dissolved in deionized/filtered drinking water containing 0.05% Tween 80 as an emulsifying agent. Verification by gas chromatography with flame ionization detection of the concentration of TCEN in pilot batches of drinking water formulations at room temperature, in drinking water bottles on empty rat cages, indicated that despite the presence of 1,4-dioxane as a stabilizing agent, the TCEN mixtures were still relatively unstable. TCEN concentration decreased to 82–90% of the nominal concentration after 72 hr (George *et al.*, 1987a). Therefore, each batch of drinking water formulation was administered to the animals for 2–3 days, the shortest possible period that could be scheduled within the study design. Formulations were mixed at 4, 15, and 40 ppm TCEN to allow the concentration of TCEN in the formulations to approach the target concentrations of 3, 10, or 30 ppm during the period of administration. A deionized/filtered water control and a vehicle control containing 0.05% Tween 80 and 0.9 ppm 1,4-dioxane,[12] the stabilizing agent found in the bulk supply of TCEN, were also used. Nevertheless, due to the volatility of the compound, predosing concentrations of TCEN ranged from 85 to 137% of the nominal concentration, although 81% of the assayed formulations were within 90–110% of

[3] This study was conducted in accordance with the Food and Drug Administration's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (FDA, 1978). Copies of the final study report (in two parts: George *et al.*, 1987a,b) are available from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22151 [NTIS Accession Nos. PB88131321/AS (Part I) and PB88134101 (Part II)].

[4] Charles River Laboratories, Inc., Raleigh, NC.

[5] Laboratory Products, Garfield, NJ.

[6] Laboratory Products, Rochelle Park, NJ.

[7] Ancare Corporation, Manhasset, NJ.

[8] Purina Certified Rodent Chow (5002), Ralston Purina Company, St. Louis, MO.

[9] Datapod Model DP220 Electronic Hygrothermograph, Omnidata International, Inc., Logan, UT.

[10] Obtained by Research Triangle Institute from the manufacturer, Aldrich Chemical Company, Milwaukee, WI (Lot No. 8725 LL).

[11] Purity determinations were conducted at Research Triangle Institute (RTI), Research Triangle Park, NC (NTP/NIEHS Contract N01-ES-45061) and involved mass, infrared, and nuclear magenetic resonance spectrometry, Karl Fischer titration, and gas chromatography. Identity and purity were confirmed at RTI (NTP/NIEHS Contract N01-ES-55080) using infrared spectrometry and gas chromatography, respectively, following completion of the study.

[12] Obtained by Research Triangle Institute from the manufacturer, Aldrich Chemical Company, Milwaukee, WI (Lot No. C 2514 KM).

the nominal concentration. Postdosing concentrations of TCEN ranged from 30 to 108% of the predosing concentration. To estimate average TCEN consumption, time-weighted averages of the TCEN concentration in the dosing solutions were calculated based on the predosing concentrations and the decay curves for the 15- and 40-ppm formulations (George *et al.*, 1987a).

Male and female rats (more than 30 per sex per group) were exposed to TCEN for 14 days prior to cohabitation and throughout cohabitation (up to 13 days). This exposure scheme was adopted to allow mating of the required number of females (total of 117 sperm-positive females) within a reasonable amount of time, while still providing approximately the same mean exposure period used by Dapson *et al.* (1984). Sperm-positive females (24–30 per group) continued to be exposed through PND 21 as in the Dapson *et al.* study (1984).

Observations. Animals were observed daily for clinical signs of toxicity. Food and water consumption and body weight were determined on selected days during the premating, gestational, and postnatal periods, but not during cohabitation. Blood samples were taken from the vena cava from sentinel male and female rats (two/sex/dose group/cohort) prior to cohabitation, to determine the premating levels of TCEN in whole blood. Cohabitation continued until 24 to 29 sperm-positive females were identified in each dose group. Male breeder rats and sperm-negative female rats were killed after the cohabitation period was completed, and blood samples were taken from the vena cava of one or two animals from each treatment group in each cohort for the determination of postcohabitation level of TCEN in whole blood.

Dams were maintained with their own litters throughout lactation to PND 21. During the postnatal period, the dams were weighed and food and water consumption was determined on PND 0, 7, 14, and 21. Individual litters were evaluated for the length of the gestational period and litter size. Pups were weighed on PND 1 and 4. On PND 4, litter size was noted, and perinatal mortality was determined (Oser and Oser, 1956). Litters containing more than 10 pups were culled to a litter size of 10, giving attention to equal sex distribution, if possible. Litters with less than 10 pups remained in the study undisturbed. Culled pups were sacrificed by ip injection of T-61 Euthanasia Solution[13] and examined for visceral malformations (Staples, 1974; Stuckhardt and Poppe, 1984). Growth and survival of the remaining pups were evaluated to PND 21, when the pups were killed by ip injection with T-61 Euthanasia Solution. Dams were anesthetized with CO_2 and then killed either by exsanguination or cervical dislocation. Blood samples were taken from the vena cava of selected dams and up to two male and two female pups in at least four litters per dose group per cohort for the determination of TCEN levels in whole blood. Each pup was necropsied, with special attention to the heart and surrounding vasculature. The uterus of each dam was stained to reveal the number of implantation sites (Salewski, 1964).

Blood analysis. Samples from adult animals were collected directly from the vena cava into vacuum tubes and immediately placed on ice. Pup samples were collected by cardiac puncture into a syringe, and then transferred to a vacuum tube and put on ice. The iced samples were transported to the analytical laboratory where they were extracted with isooctane. All blood samples were extracted on the day they were collected. In addition, to account for possible loss of TCEN from the stored extracts and variation in instrument response, on each day samples were received, spiked control blood samples were extracted and the extracts stored as a set with the study sample extracts. The control and sample extracts were stored under refrigeration and analyzed in sets for TCEN at a later time, using gas chromatography with electron capture detection.

Statistical analyses. Analysis of selected data was carried out using the general linear model (GLM) procedure in the SAS software library (SAS Institute, Inc., 1982a,b). Prior to analysis, an arcsine–square root transformation was performed on all litter-derived percentage data (Snedecor and Cochran, 1967), and Bartlett's test for homogeneity of variance was performed on all data to be analyzed by ANOVA (Winer, 1962). Dose–response relationships for selected measures were evaluated using a test for linear trend. Analysis of variance (ANOVA) was used to determine whether significant dose effects had occurred. When ANOVA revealed significant differences among groups, then Dunnett's test (Dunnett, 1955, 1964) and Williams' test (Williams, 1971, 1972) were used to compare each TCEN-treated group with the vehicle control group for that measure ($\alpha = 0.05$). Nominal scale measures were analyzed by a test for linear trend on proportions, and a χ^2 test for independence among treatment groups (Siegel, 1956). When χ^2 revealed significant ($p < 0.05$) differences among groups, then a one-tailed Fisher exact probability test ($\alpha = 0.05$) was used for pairwise comparisons between each TCEN-treated group and the vehicle control. In addition, for all data analyzed by parametric statistics, a comparison between the deionized/filtered water control and the vehicle control group was conducted using Student's t test (SAS Institute Inc., 1982b). Nonparametric statistics were used to analyze selected data that violated the assumptions of the parametric tests. The Kruskal–Wallis (Siegel, 1956), Mann–Whitney U (Siegel, 1956), and Jonckheere (Jonckheere, 1954) tests were used to examine the experimentwise effect of dose, the pairwise effect of dose, and the dose–response trend, respectively, when data were continuous or approximately continuous but not normally distributed. In addition, for all such data, a comparison between the deionized/filtered water control and the vehicle control group was conducted using the Mann–Whitney U test.

[13] American Hoechst, Somerville, NJ.

TABLE 1

POSTNATAL EVALUATION: WATER CONSUMPTION BY MALE AND FEMALE CD RATS EXPOSED TO 1,1,1-TRICHLOROETHANE

	Water consumption (g/kg/day)				
	Controls				
	Deionized/ filtered water	0.05% Tween 80 + 0.9 ppm 1,4-dioxane	1,1,1-Trichloroethane[a]		
			3 ppm	10 ppm	30 ppm
Premating Day 1 to mating Day 1 (14 days)[b]					
Males[c]	96.7 ± 3.1	96.0 ± 3.0	98.5 ± 3.6	92.8 ± 2.8	88.1 ± 2.1
Females[c]	118.1 ± 3.6	119.3 ± 3.0	115.7 ± 3.2	116.5 ± 3.1	109.1 ± 2.5
GD 0 to parturition[d]	134.5 ± 4.7	134.4 ± 4.1	128.1 ± 3.9	135.4 ± 4.4	129.0 ± 4.3
PND 1 to 21[e]	237.3 ± 5.9	222.0 ± 7.6	222.9 ± 8.1	232.1 ± 4.3	218.5 ± 6.9

[a] Target concentration.
[b] Includes all animals assigned to study; means ± SEM.
[c] Test for linear trend involving the Tween 80 control group and the TCEN-treated groups; $p < 0.05$.
[d] Includes all confirmed-pregnant dams delivering live litters; means ± SEM.
[e] Includes all dams with litters surviving to PND 21; means ± SEM.

RESULTS

No deaths, morbidity, or distinctive clinical signs were exhibited by either the male or the female breeder rats during the premating or cohabitational periods. For males, there was no significant effect of treatment on body weight, body weight gain, or food consumption during the premating period. Premating water consumption (g/kg/day) for male breeders exhibited a decreasing trend, involving primarily the 30-ppm TCEN group, suggesting a slight aversion to the TCEN-treated water at the 30-ppm dose level (Table 1). Average calculated TCEN consumption for males during the premating period, based on time-weighted averages of TCEN concentration, was 0.3, 0.9, and 2.6 mg/kg/day for 3-, 10-, and 30-ppm groups, respectively.

For females during the premating period, there was no effect of treatment on body weight, body weight gain, or food consumption. Water consumption exhibited a decreasing trend suggesting that the females also disliked the TCEN-treated water (Table 1). Mean TCEN consumption during the premating period for breeder females, based on time-weighted averages of the concentration of TCEN in the 3-, 10-, and 30-ppm TCEN groups was calculated to be 0.3, 1.3, and 3.3 mg/kg/day, respectively.

During the gestational period, no maternal deaths, morbidity, or distinctive clinical signs were observed. There was no effect of treatment on body weight, body weight gain, food consumption, or water consumption (Table 1). Mean maternal TCEN intake from GD 0 to parturition based on time-weighted averages of the TCEN concentration in the dosing formulations indicated that females in the 3-, 10-, and 30-ppm TCEN dose groups received an estimated 0.3, 1.2, and 3.5 mg/kg/day, respectively.

For dams delivering live litters, there was no effect of treatment on maternal body weight, maternal body weight gain, or food or water consumption during the lactational period (PND 1–21). Neither was there any

TABLE 2

POSTNATAL EVALUATION: REPRODUCTIVE OUTCOME IN CD RATS EXPOSED TO 1,1,1-TRICHLOROETHANE FROM PREMATING TO POSTNATAL DAY 21

	Controls		1,1,1-Trichloroethane[a]		
	Deionized/ filtered water	0.05% Tween 80 + 0.9 ppm 1,4-dioxane	3 ppm	10 ppm	30 ppm
Percentage confirmed-pregnant dams with live litters	96.0 (24/25)	100.0 (22/22)	100.0 (24/24)	100.0 (25/25)	100.0 (22/22)
Number of implantation sites per dam[b,c]	15.5 ± 0.3	14.3 ± 1.2	16.6 ± 0.8	15.5 ± 0.7	15.6 ± 0.7
Length of gestation (days)[b,c]	21.5 ± 0.1	21.3 ± 0.1	21.5 ± 0.1	21.4 ± 0.1	21.5 ± 0.1
Number of live pups per litter on PND 1[b,c]					
PND 1[b,c]	14.4 ± 0.3	13.9 ± 0.9	15.0 ± 0.7	14.2 ± 0.5	13.4 ± 0.7
PND 4[b,c]	14.1 ± 0.3	13.4 ± 0.9	14.5 ± 0.7	13.8 ± 0.5	12.9 ± 0.9
Percentage mortality[b,c]					
Implantation through PND 1[d]	7.7 ± 1.9	4.9 ± 1.5	9.3 ± 2.2	7.2 ± 1.8	15.9 ± 3.2*
Implantation through PND 4[e]	9.7 ± 2.2	14.5 ± 5.5	12.4 ± 2.4	9.7 ± 2.1	19.6 ± 4.7
Average pup body weight (g)[b,c]					
PND 1	6.0 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.0 ± 0.1
PND 21	40.3 ± 0.8	41.8 ± 1.3	40.2 ± 0.8	40.8 ± 0.7	40.4 ± 1.1

[a] Target concentration.
[b] n = 18–25 for each group, since some data were inadvertently not collected or not available.
[c] Presented as means ± SEM.
[d] Jonckheere's test, involving the Tween 80 control group and the TCEN-treated groups; $p \leq 0.05$.
[e] Jonckheere's test, involving the Tween 80 control group and the TCEN-treated groups; $p \leq 0.01$.
* Mann–Whitney U test, comparing the TCEN-treated group with the Tween 80 control group; $p \leq 0.05$.

effect on the number of implantation sites per dam or the length of the gestational period (Table 2). When the vehicle control group was compared with the TCEN-treated groups, the only significant effect noted was an increase in the percentage mortality from implantation to PND 1 due solely to a significant increase above the vehicle control at 30 ppm TCEN. This increase appeared to be due primarily to 61% mortality in one 30-ppm TCEN-treated litter on PND 1. There was no effect of treatment on the number of live pups per litter on PND 1 or 4, or average pup body weight on PND 1 or 21. In addition, there was no significant difference between the deionized/filtered water group and the vehicle control group for any measure of pup toxicity (Table 2). There was no effect of treatment on maternal water consumption (Table 1). Mean maternal TCEN consumption from PND 1 to 21, based on the time-weighted average of the concentration of TCEN in the formulations, was estimated to be 0.6, 2.0, and 5.9 mg/kg/day for the 3-, 10-, and 30-ppm TCEN dose groups, respectively.

Visceral examination of pups culled on PND 4 indicated that out of a total of 482 pups examined, only one (3-ppm dose group) had patent ductus arteriosus. Of those pups found dead on PND 1, a total of 10 pups in

TABLE 3

SUMMARY OF MALFORMATIONS OBSERVED ON POSTNATAL DAY 21 IN CD RAT PUPS EXPOSED TO 1,1,1-TRICHLOROETHANE FROM CONCEPTION TO POSTNATAL DAY 21[a]

	Controls		1,1,1-Trichloroethane[b]		
	Deionized/ filtered water	0.05% Tween 80 + 0.9 ppm 1,4-dioxane	3 ppm	10 ppm	30 ppm
Number of pups examined	238	203	229	246	204
Number of litters examined	24	21[c]	24	25	21[c]
Number of pups with malformations	1	2	2	1	0
Number of litters with one or more malformed pups	1	2	2	1	0
Number of pups with external malformations					
(no tail)	0	1	1	0	0
Number of pups with visceral malformations					
Hydronephrosis	1	0	1	1	0
Small kidney	0	1	0	0	0

[a] No significant treatment-related effects were noted.
[b] Target concentration.
[c] All pups in one litter were missing and presumed dead on pnd 4.

the TCEN-treated groups (6 from four litters at 3 ppm; 1 at 10 ppm; 3 from two litters at 30 ppm) were observed upon visual examination to have patent ductus arteriosus, with no occurrence of patent ductus arteriosus in either the deionized/filtered water group or the vehicle control group. Statistical analysis of the incidence by litter of patent ductus arteriosus indicated no significant effect of treatment on this parameter. Patent ductus arteriosus was not observed in any pup that died between PND 2 and 21.

When pups were evaluated for external and visceral malformations on PND 21, there was no evidence of cardiac malformation in any dose group (Table 3). An external malformation (no tail) was observed in one pup in each of the vehicle control (1/203) and 3-ppm (1/229) TCEN groups, hydronephrosis was observed in one pup in the deionized/filtered water group (1/238) and the 3-ppm (1/229) and 10-ppm (1/246) TCEN groups, and small kidney was observed in one pup (1/203) in the vehicle control group (Table 3).

Blood samples from a total of 60 animals (6 males and 6 females per dose group) after the premating exposure, 45 animals (5 or 6 males and 2–6 females per dose group) after the postcohabitational exposure, and 61 dams (12–14 per dose group) and 127 pups (up to 2 male and 2 female pups from each of their litters) on PND 21 were analyzed. More than 90% of the samples analyzed for each period contained TCEN at or slightly below the limit of detection (5.0 ng/ml). Validation of the analytical method indicated that had the compound been consistently present in the calibrated range (5–50 ng/ml) it would have been detected.

DISCUSSION

In the present study, TCEN administered in the drinking water at target concentrations of 0, 3, 10, or 30 ppm to CD rats caused no significant toxicity in male and female breeders after a 14-day premating exposure, and had no effect on measures of maternal toxicity during the gestational period. Measures of food and water consumption throughout the study indicated that the TCEN-dosed drink-

ing water was well tolerated by both male and female animals, although water consumption was slightly decreased at 30 ppm, suggesting decreased palatability at the high dose. In addition, measurements for the vehicle control and deionized/filtered water groups indicated that the two control groups were comparable, with only very minor differences for maternal body weight or water consumption. TCEN had no effect on fertility, length of gestational period, live litter size, pup body weight, or pup survival from PND 1 to 21. A slight but significant increase in the mortality from implantation to PND 1 was observed in the 30-ppm TCEN dose group. The occurrence of this effect may have been influenced by a high mortality in one treated litter; however, since PND 1 litter size was not significantly affected, and no indication of compromised prenatal survival had been observed in the teratology study, the significance of this result is unclear. There was clearly no indication of an increase in the incidence of cardiac malformations or visceral malformations of any other organ system in pups on either PND 4 or 21, even at concentrations as high as 30 ppm TCEN. This is in contrast with the results of Dapson *et al.* (1984), who indicated a significant increase in persistent ductus arteriosus and other cardiac malformations in surviving PND 21 offspring exposed to 10 ppm TCEN in the drinking water. In the present study, patent ductus arteriosus was observed in 10 TCEN-treated pups (3, 10, or 30 ppm) found dead on PND 1 and in 1 pup (3 ppm) culled on PND 4. Statistical analysis of these data did not confirm a treatment effect. In addition, studies of the time course of postnatal closure of the ductus arteriosus in rats indicate that a functional closure of the vessel (i.e., constriction of the smooth muscle wall) occurs within the first 1–3 hr of birth (Hornblad, 1969). This constriction may be maintained by prostaglandins or by the increased oxygen tension in the blood, and has been shown to be reversible at this stage (Hornblad, 1969). The second stage of closure of the ductus arteriosus involves permanent sealing of the lumen of the vessel, and may take up to 5 days in the rat (Heymann and Rudolph, 1975). For those pups found dead on PND 1, observation of a patent ductus arteriosus may have been the result of the absence, after death, of chemical (prostaglandin or oxygen) control of construction of the vessel. Patent ductus arteriosus was observed only in one living pup on PND 4, i.e., during the postnatal period when constriction of the ductus is still reversible (Hornblad, 1969). Observation of a patent ductus arteriosus in this animal may have been the result of a lowered oxygen concentration in the vessel, due to cyanosis caused by the method of euthanasia. Therefore, the presence of patent ductus arteriosus in PND 1 or 4 animals as observed in this study was not attributed to TCEN exposure; however, further experimentation may be warranted to fully elucidate the origin of this observation.

The lack of detection of TCEN in the blood of animals in the postnatal study was surprising in light of the results reported by Dapson *et al.* (1984 and personal communication); however, subsequent kinetic modeling of TCEN after ingestion in drinking water indicated that detection of TCEN in blood using the present study design was unlikely, as explained below.

TCEN is rapidly absorbed from both the lungs and the gastrointestinal system (Holmberg *et al.*, 1977; Stewart, 1968). The compound is then rapidly redistributed from the blood into the tissues. The majority of the systemically absorbed TCEN is eliminated via the lungs (Hake *et al.*, 1960). Kinetic modeling of the disposition of TCEN in the postnatal study female breeders at the end of the premating period, based on time-weighted levels of TCEN in the drinking water (see Appendix), suggested that based on measured water consumption, the highest blood levels that could be expected in these animals at the time of sacrifice would be 0.51, 1.57, and 4.73 ng/ml blood. In actuality, with staggered times of sacrifice, the blood TCEN levels would likely be less. Based upon the

vailable pharmacokinetic information on TCEN, it seems unlikely that the TCEN concentration in the blood of the animal would exceed the 5 ng/g limit of detection, using the exposure regimen and sacrifice schedule in the present study. Since relative water consumption (i.e., based on body weight) for male breeders at the end of the premating period was actually less than for female breeders, this pattern would be expected to hold true for the male rats as well, in addition to being indicative of blood TCEN levels at the end of the cohabitation period. A kinetic model for TCEN disposition during lactation was not developed in the present study; however, it might be assumed that although relative water consumption for lactating animals is greater than for their nonlactating counterparts, the lactating females would exhibit steady-state levels of TCEN that were similar to those of nonlactating females, and that the remainder of the TCEN consumed would be evenly distributed through the milk to the dam's litter. Alternatively, TCEN may be preferentially distributed through the milk due to high lipid solubility of the test compound. Further experiments may be warranted to define the disposition of orally administered TCEN in the pregnant rodent.

The results of the present study and the companion teratology study (George *et al.*, 1987a,b) support the conclusions of Lane *et al.* (1982) and Schwetz *et al.* (1975), who showed that TCEN was not a reproductive toxicant or teratogen even at doses 300- to 1300-fold higher; however, our results are in contrast with the preliminary results of Dapson *et al.* (1984) both with regard to the induction of malformations in exposed offspring and with respect to the measured blood levels of TCEN. Data from the present study support the conclusion that, under the conditions of the study, exposure to TCEN does not present a selective risk to the developing organism, and does not induce cardiac or other malformations.

APPENDIX

Pharmacokinetic Modeling of Blood Levels of TCEN

To clarify the reason for the low TCEN values obtained in the blood analysis, a pharmacokinetic model of TCEN in the blood after administration in the drinking water was generated using data from the premating period of the postnatal evaluation.

Standard formulas for a two-compartment open model with oral absorption were used (Wagner, 1975). Pharmacokinetic parameter estimates for the distribution and elimination of TCEN in rats after exposure by inhalation were obtained from Schumann *et al.* (1982). Values for the bioavailability (f) and the first-order absorption rate constant (k_a) for the oral route of exposure were estimated to be 0.50 and 1.0 min^{-1}, respectively (Dallas *et al.*, 1987). To simulate the intermittent nature of the drinking behavior of rats (Armstrong, 1980), 28% of the daily dose was divided into 24 equal parts to represent potential consumption outside feeding periods. The remaining 72% was divided evenly between two nocturnal feeding periods evenly spaced during the dark cycle. Since the dark cycle in the present study was from 7 PM to 7 AM these feeding periods were estimated to be at 11 PM and 3 AM. Simulations were performed using SAS software running on an IBM 3081 mainframe computer at Triangle Universities Computation Center (TUCC), Research Triangle Park, North Carolina.

Based on estimates of TCEN consumption, the drinking habits of rats, and other data describing the kinetic behavior of TCEN, maximum blood levels of TCEN were predicted for females after the premating exposure. At 9:00 AM, the earliest time of sacrifice, animals receiving the 3-, 10-, and 30-ppm formulations would have blood TCEN levels of approximately 0.51, 1.57, and 4.73 ng/g, respectively. Subsequently, the blood levels would fluctuate around the steady-state levels for each treatment group,

i.e., 0.33, 1.04, and 3.13 ng/g blood for the 3-, 10-, and 30-ppm groups, respectively. When a "worst case" was simulated for the 30-ppm group, i.e., the entire daily dose of TCEN consumed at 7:00 AM, and the animals were all killed at 9:00 AM, the TCEN blood level was predicted to be 23.64 ng/g blood at 9:00 AM, decreasing to 7.06 ng/g blood by 1:00 PM, the last possible time of sacrifice.

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REFERENCES

ACGIH (1987). *Threshold Limit Values and Biological Exposure Indices for 1987–1988.* American Conference of Governmental Industrial Hygienists, Cincinnati, OH.

ARMSTRONG, S. (1980). A chronometric approach to the study of feeding behavior. *Neurosci. Biobehav. Rev.* **4**, 27–53.

CDHS (1982). *Pregnancy Outcomes in Santa Clara County, 1980–1982. Summaries of Reports of Two Epidemiological Studies.* California Department of Health Services, Epidemiological Studies Section, Berkley.

C&E News (1987). Facts and figures for the chemical industry. *Chem. Eng. News* **65**, 24–76.

DALLAS, C. E., RAMANATHAN, R., MURALIDHARA, S., GALLO, J. M., AND BRUCKNER, J. V. (1987). Relative uptake and elimination of 1,1,1-trichloroethane (TRI) in inhalation and oral exposures in rats. *Toxicologist* **7**, 269.

DAPSON, S. C., HUTCHEON, D. E., AND LEHR, D. (1984). Effect of methyl chloroform on cardiovascular development in rats. *Teratology* **29**, 25A.

DUNNETT, C. W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Amer. Stat. Assoc.* **50**, 1096–1121.

DUNNETT, C. W. (1964). New tables for multiple comparisons with a control. *Biometrics* **20**, 482–491.

FDA (Dec. 22, 1978). Food and Drug Administration good laboratory practice regulations for nonclinical laboratory studies. *Fed. Regist.* **43**(247), 60013–60025.

GEORGE, J. D., PRICE, C. J., MARR, M. C., MORRISSEY, R. E., AND SCHWETZ, B. A. (1987a). *Developmental Toxicity Evaluation of 1,1,1-Trichloroethane (CAS No. 71-55-6) Administered to CD Rats. Part I. Postnatal Evaluation, Final Study Report.* NTP/NIEHS Contract N01-ES-55080, Aug. 28, 1987. NTIS Accession No. PB88131321/AS.

GEORGE, J. D., PRICE, C. J., MARR, M. C., MORRISSEY, R. E., AND SCHWETZ, B. A. (1987b). *Developmental Toxicity Evaluation of 1,1,1-Trichloroethane (CAS No. 71-55-6) Administered to CD Rats. Part II. Teratological Evaluation, Final Study Report.* NTP/NIEHS Contract N01-ES-55080, Sept. 23, 1987. NTIS Accession No. PB88134101.

HAKE, C. L., WAGGONER, T. B., ROBERTSON, D. N., AND ROWE, V. K. (1960). The metabolism of 1,1,1-trichloroethane by the rat. *Arch. Environ. Health* **1**, 101–105.

HEYMANN, M. A., AND RUDOLPH, A. M. (1975). Control of the ductus arteriosus. *Physiol. Rev.* **55**, 62–78.

HOLMBERG, B., JAKOBSON, I., AND SIGVARDSSON, K. (1977). A study on the distribution of methylchloroform and *n*-octane in the mouse during and after inhalation. *Scand. J. Environ. Health* **3**, 43–52.

HORNBLAD, P. Y. (1969). Experimental studies on closure of the ductus arteriosus utilizing whole body freezing. *Acta Paediat. Scand. Suppl.* **190**, 6–21.

IARC (1979). Evaluation of the carcinogenic risk of chemicals to humans (1,1,1-trichloroethane). *IARC Monogr.* **20**, 515–531.

JONCKHEERE, A. R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133–145.

LANE, R. W., RIDDLE, B. L., AND BORZELLECA, J. F. (1982). Effects of 1,2-dichloroethane and 1,1,1-trichloroethane in drinking water on reproduction and development in mice. *Toxicol. Appl. Pharmacol.* **63**, 409–421.

OSER, B. C., AND OSER, M. (1956). Nutritional studies on rats on diets containing high levels of partial ester emulsifiers. II. Reproduction and lactation. *J. Nutr.* **60**, 489–505.

SAS Institute Inc. (1982a). *SAS User's Guide: Basics,* 1982 ed. SAS Institute Inc., Cary, NC.

SAS Institute Inc. (1982b). *SAS User's Guide: Statistics,* 1982 ed. SAS Institute Inc., Cary, NC.

SALEWSKI, E. (1964). Farbemethode Zum Makroskopischen Nachweis Von Implantations Stellen Am

Uterus Der Ratte. *Naunyn-Schmiedeberg's Arch. Exp Pathol. Pharmakol.* **247**, 367.

SCHUMANN, A. M., FOX, T. R., AND WATANABE, P. G. (1982). [^{14}C]Methyl Chloroform (1,1,1-Trichloroethane): Pharmacokinetics in rats and mice following inhalation exposure. *Toxicol. Appl. Pharmacol.* **62**, 390–401.

SCHWETZ, B. A., LEONG, B. K. J., AND GEHRING, P. K. (1975). The effect of maternally inhaled trichloroethylene, perchloroethylene, methyl chloroform, and methylene chloride on embryonal and fetal development in mice and rats. *Toxicol. Appl. Pharmacol.* **32**, 84–96.

SIEGEL, S. (1956). *Nonparametric Statistics for the Behavioral Sciences.* McGraw–Hill, New York.

SNEDECOR, G. W., AND COCHRAN, W. G. (1967). *Statistical Methods.* 6th ed. Iowa State Univ. Press, Ames.

STAPLES, R. E. (1974). Detection of visceral alterations in mammalian fetuses. *Teratology* **9**, 7.

STEWART, R. D. (1968). The toxicology of 1,1,1-trichloroethane. *Ann. Occup. Hyg.* **11**, 71–79.

STUCKHARDT, J. L., AND POPPE, S. M. (1984). Fresh visceral examination of rat and rabbit fetuses used in teratogenicity testing. *Teratogen. Carcinogen. Mutagen.* **4**, 181–188.

WAGNER, J. G. (1975). *Fundamentals of Clinical Pharmacokinetics.* Drug Intelligence Publications, Inc., Hamilton, IL.

WILLIAMS, D. A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103–117.

WILLIAMS, D. A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519–531.

WINER, B. J. (1962). *Statistical Principles in Experimental Design.* McGraw–Hill, New York.