National Toxicology Program
Toxicity Report Series
Number 18

NTP Technical Report
on Toxicity Studies of

Methyl Ethyl Ketone Peroxide

(CAS No. 1338-23-4)

in Dimethyl Phthalate

(CAS No. 131-11-3)

(45:55)

Administered Topically
to F344/N Rats and B6C3F$_1$ Mice

Errol Zeiger, PhD, Study Scientist
National Toxicology Program
Post Office Box 12233
Research Triangle Park, NC 27709

NIH Publication 93-3341
February 1993

United States Department of Health and Human Services
Public Health Service
National Institutes of Health

Foreword

The National Toxicology Program (NTP) is made up of four charter agencies of the United States Department of Health and Human Services (DHHS):

- the National Cancer Institute (NCI) of the National Institutes of Health;
- the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health;
- the National Center for Toxicological Research (NCTR) of the Food and Drug Administration; and
- the National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control.

In July 1981, the Carcinogenesis Bioassay Testing Program was transferred from NCI to NIEHS. NTP coordinates the relevant Public Health Service programs, staff, and resources that are concerned with basic and applied research and with biological assay development and validation.

NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

To carry out its mission, NTP designs and conducts studies to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's toxic potential.

The studies described in this toxicity study report were performed under the direction of NIEHS and were conducted in compliance with NTP laboratory health and safety requirements. These studies met or exceeded all applicable federal, state, and local health and safety regulations. Animal care and use were in accord and compliance with the Public Health Service Policy on Humane Care and Use of Animals.

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This NTP report on the toxicity studies of methyl ethyl ketone peroxide is based primarily on 2-week studies that took place in October 1986 and 13-week studies that began in July 1987 and ended in October 1987 at Hazleton Laboratories America, Inc., Rockville, MD.

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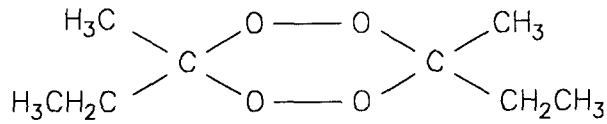
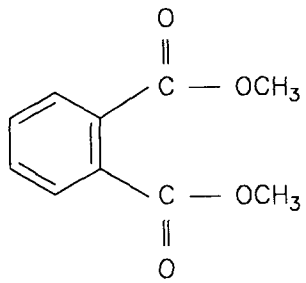


METHYL ETHYL KETONE PEROXIDE

Molecular Formula	$C_8H_{16}O_4$
CAS Number	1338-23-4
Molecular Weight	176.216
Synonyms	2-Butanone peroxide



DIMETHYL PHTHALATE

Molecular Formula	$C_{10}H_{10}O_4$
CAS Number	131-11-3
Molecular Weight	194.19
Synonyms	1,2-Benzenedicarboxylic acid dimethyl ester; phthalic acid dimethyl ester; methyl phthalate; dimethyl 1,2-benzene-dicarboxylate; DMP

Methyl ethyl ketone peroxide (MEKP) is an unstable organic peroxide used in the manufacture of acrylic resins, as a hardening agent for fiberglass-reinforced plastics, and as a curing agent for unsaturated polyester resins. It is commercially available as a 40% to 60% solution in dimethyl phthalate (DMP). Because exposure to MEKP is typically through dermal contact, 2-week and 13-week toxicity studies were conducted by topical application of MEKP in DMP (45:55 w/w) to the clipped dorsal region of male and female Fischer 344/N rats and B6C3F$_1$ mice. Animals were evaluated for histopathology and for reproductive endpoints. *In vitro* genetic toxicity studies of MEKP included assessments of mutagenicity in *Salmonella typhimurium* and in mouse lymphoma L5178Y cells and analysis of chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells. In addition, the peripheral blood of mice from the 13-week study was evaluated in the micronucleus assay.

In the 2-week studies, groups of 5 animals of each species and sex were administered MEKP in DMP for 5 days per week at doses of 50.6, 101.3, 202.5, 405, and 810 mg/kg body weight per day for rats and 112.5, 225, 450, 900, and 1800 mg/kg body weight per day for mice. Control groups received DMP or no treatment. No rats died during the studies, but at least 1 mouse in each group receiving MEKP died. Body weight gains of rats decreased with increasing doses of MEKP; body weight gains of mice were not affected by treatment. The primary effects of topical administration of MEKP in both rats and mice were an extensive coagulative necrosis of the epidermis and dermis, variable degrees of inflammation of the adnexa, and epidermal regeneration and hyperplasia at the application site. Lesions considered secondary to the dermal lesions included increased hematopoiesis in the spleen in rats and mice and increased myeloid hyperplasia of the bone marrow in mice, primarily at the higher doses. Mice showed a marked, dose-related increase in liver weight.

In the 13-week dermal studies, groups of 10 rats and 10 mice of each sex were administered MEKP in DMP for 5 days per week at doses of 1.07, 3.57, 10.7, 35.7, and 107 mg/rat and 0.357, 1.19, 3.57, 11.9, and 35.7 mg/mouse. All high-dose mice, 3 high-dose female rats, and 1 female mouse in the 11.9 mg/animal group died or were sacrificed during the first week of the studies. Skin lesions similar to those seen in the 2-week studies were judged of sufficient severity to warrant early termination of surviving

rats and mice in the 2 highest dose groups. All rats and mice in the remaining dose groups survived to the end of the studies, and weight gains were generally lower with increasing doses of MEKP. Skin lesions at the application site for the remaining animals (rats and mice) in the 10.7 mg/rat and 3.57 mg/mouse dose groups involved a spectrum of necrosis, inflammation, and acanthosis (epidermal hyperplasia). Lesions in the lower dose groups were limited to acanthosis and hyperkeratosis in rats (1.07 and 3.57 mg/rat) and acanthosis in mice (0.357 and 1.19 mg/mouse). While splenic and bone marrow lesions similar to those described in the 2-week studies were seen in animals that died early in the 13-week studies and in the rats and mice that showed ulcerative or necrotic injury, no other systemic changes were noted in animals that did not show ulcerative skin lesions.

In genetic toxicity studies, MEKP in DMP (45:55 w/w) was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98, with or without S9 activation. A positive response was obtained in the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y cells without S9. In cytogenetic tests with Chinese hamster ovary cells, MEKP induced sister chromatid exchanges and chromosomal aberrations, with and without S9. No increase in the frequency of micronucleated erythrocytes was observed in peripheral blood samples obtained from male and female mice at the termination of the 13-week toxicity study.

In summary, topical administration of MEKP in DMP resulted in a spectrum of necrotic, inflammatory, and regenerative skin lesions limited to the application site. Histopathologic changes in the spleen and bone marrow were also seen in rats and mice with ulcerative skin lesions, and were considered a secondary response. A no-observed-adverse-effect level (NOAEL) for histopathologic skin lesions could not be determined from these studies, as lesions were observed with administration of daily doses as low as 1.07 mg for rats and 0.357 mg for mice.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies of methyl ethyl ketone peroxide on June 24, 1992 are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members determine if the design and conditions of these NTP studies are appropriate and ensure that this toxicity study report presents the experimental results and conclusions fully and clearly.

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SUMMARY OF PEER REVIEW COMMENTS

On June 24, 1992, the Technical Reports Review Subcommittee of the Board of Scientific Counselors for the National Toxicology Program Met in Research Triangle Park, NC, to review the draft technical report on toxicity studies of methyl ethyl ketone peroxide (MEKP) in dimethyl phthalate.

Dr. Errol Zeiger, NIEHS, introduced the short-term toxicity studies of MEKP by reviewing the uses of the chemical, study rationale, experimental design, and results.

Dr. Davis, a principal reviewer, said that this was a well-written and concise report. He asked that the abstract clarify which of the high-dose mice died and which were killed moribund because of extensive skin lesions, and also asked that the bone marrow lesions seen in rats be mentioned in the abstract. He also asked for clarification of statements alluding to the significant potential for human exposure to MEKP. Dr. Zeiger agreed to modify the abstract as requested. He stated that clear potential for human exposure to MEKP exists based on use patterns, and because MEKP is one of the more reactive peroxides, it was considered a good candidate for study.

Dr. Hayden, a second principal reviewer, thought the study appeared well done, although the 2 highest doses selected for mice were excessive, as was stated in the report.

Dr. Silbergeld did not know whether peroxides have been demonstrated to be neurotoxins, but wondered whether the clinical examinations of the animals were sufficient to rule out such a possibility. Dr. R.A. Griesemer, NIEHS, replied that cageside observations are just stage 1 of a 3-tiered approach to neurotoxicity testing, and that a decision to proceed to the next tier is made according to the presence of abnormal clinical signs and the presence of possible dose-related histologic lesions.

After discussion of editorial matters, Dr. Carlson accepted the report on behalf of the peer review panel.

INTRODUCTION

Physical Properties, Production, Use, and Exposure

Methyl ethyl ketone peroxide (MEKP) is a colorless liquid with a flash point of 125° to 200°F. The pure chemical is an unstable peroxide, capable of releasing molecular oxygen. It is shock, sunlight, and heat sensitive, and undergoes explosive decomposition at 230°F. Commercial MEKP contains a mixture of peroxide, hydroperoxide, and active oxygen in dimethyl phthalate (DMP), which is used as a diluent to prevent decomposition and explosion of MEKP. Because of the high reactivity of MEKP, it is available only as a 40% to 60% solution in dimethyl phthalate or other phthalates. MEKP can undergo spontaneous ignition or decomposition if mixed with readily oxidizable organic or flammable materials or chemical reactants.

MEKP is used in the manufacture of acrylic resins, as a hardening agent for fiberglass-reinforced plastics, and as a curing agent for unsaturated polyester resins (Hawley, 1981). It acts through the formation of free radicals that catalyze the polymerization of the plastic monomer.

It was estimated that in the United States, 4.09×10^5 kg of MEKP were produced in 1979 and 2.68×10^6 kg were produced in 1982 (SRI, 1989). An estimated 20,000 workers may have been exposed to MEKP in 1974 (NOHS, 1974) and 25,800 workers, including 6,500 women, were potentially exposed in 1983 (NIOSH, 1990). Workplace exposure to MEKP typically arises in one of 2 ways (Purnell *et al.*, 1979). Workers are exposed to MEKP in the manufacture of decorative and structural furniture parts made of styrene polymer plastic; in this operation, a solution of styrene and MEKP is poured into molds and allowed to cure before being removed by hand (NIOSH, 1974). In the production of fiberglass-reinforced polyester resin hulls for boats, it was reported that workers were spraying the hulls with polyester resins containing free styrene monomer and MEKP (Brigham and Landrigan, 1985; NIOSH, 1988).

MEKP is regulated as an indirect food additive by the U.S. Food and Drug Administration. It is permitted as a catalyst in the production of resins, to be used at levels not to exceed

2% in the finished resin (21 CFR 177.2420). The Occupational Safety and Health Administration (OSHA) has established a ceiling concentration of MEKP of 0.2 ppm (about 1.5 mg/m^3), and a permissible exposure limit (PEL) ceiling of 0.7 ppm (about 5 mg/m^3) in the work environment (ACGIH, 1990; 29 CFR 1910.1000).

Toxicity

HUMAN EFFECTS

In the work environment, human exposure to MEKP is by inhalation of, and dermal exposure to, aerosolized MEKP during the spraying procedure used in some manufacturing processes, or by dermal exposure to the liquid substance. MEKP is highly irritating and corrosive to skin and mucous membranes. A number of cases in which people accidentally or deliberately ingested MEKP solutions, occasionally with fatal results, have been reported.

Symptoms of acute MEKP poisoning by ingestion have included gastrointestinal bleeding, abdominal burns, necrosis, perforation of the stomach, stricture of the esophagus, severe metabolic acidosis, rapid hepatic failure, rhabdomyolysis, and respiratory insufficiency (Deisher, 1958; Burger and Chandor, 1971; Wojdyla *et al.*, 1979; Mittleman *et al.*, 1986; Karhunen *et al.*, 1990). Temporary cardiac arrest (Karhunen *et al.*, 1990) and toxic myocarditis (Dines and Shipman, 1962) have also been reported. An autopsy of 1 victim showed massive periportal hepatic necrosis accompanied by atypical pseudoductular proliferation. The proliferating cells exhibited atypia and mitoses (Karhunen *et al.*, 1990). The toxic oral dose of MEKP in dimethyl phthalate was estimated to be 50 to 100 mL (Wojdyla *et al.*, 1979).

Corneal injury was seen in individuals with accidental single exposure to MEKP. Significant chronic involvement was seen only in the areas of the corneoscleral limbus and cornea. It was noted that delayed MEKP keratitis resembles delayed mustard gas keratitis by its slow progression, exacerbations and remissions, corneal hypoesthesia, and similar corneal changes. By analogy to mustard gas, the effects of MEKP may be the result of its alteration of corneal macromolecules to produce new antigens. This would result in an

autoimmune response directed at the cornea that could lead to the observed delayed keratitis (Fraunfelder *et al.*, 1990).

ANIMAL TOXICITY

A single intraperitoneal injection of MEKP in rats led to prostration, followed by death; the LD_{50} was estimated to be 65 mg/kg. The LD_{50} for a single oral gavage dose in rats was 484 mg/kg. Inhalation of MEKP for 4 hours produced an LC_{50} of 200 ppm for rats and 170 ppm for mice. Pathologic findings included hyperemia of the lungs, with petechial hemorrhages on the lung surface in some animals and gross hemorrhages in others. Nasal porphyrin exudate occurred occasionally in acute intraperitoneal and inhalation studies (Floyd and Stokinger, 1958). In rats injected with about one-fifth the LD_{50} (*i.e.*, 15 mg MEKP/kg of body weight) 3 times per week for 7 weeks, the liver was mildly damaged and showed depletion of glycogen, but showed no dissociation of liver cells (Floyd and Stokinger, 1958).

In rabbit skin tests, a single administration of MEKP to shaved skin resulted in erythema, edema, and vesiculation within 2 or 3 days. The maximal nonirritating concentration was estimated to be 1.5% peroxide (Floyd and Stokinger, 1958). No histopathology was performed in these studies. The serum albumin/globulin ratio in rabbits increased over a 2-week period following 3 cutaneous applications of MEKP per week, but a similar increase was also seen in animals treated with the diluent, dimethyl phthalate, alone (Floyd and Stokinger, 1958). In eye tests in rabbits, the maximal nonirritating concentration was estimated to be 0.6% peroxide. However, it was noted that none of the organic peroxides tested caused irritation when washed from the eyes within 4 seconds after application (Floyd and Stokinger, 1958).

A number of studies have related the toxicity of MEKP to its peroxidizing ability *in vivo*. These studies have also addressed the mechanisms by which toxicity is produced and the mitigation of MEKP toxicity by antioxidants. MEKP has been described as a more potent *in vivo* lipid-peroxidizing agent than cumene or *t*-butyl hydroperoxide (Litov *et al.*, 1981), and was the most toxic of 4 organic peroxides tested when administered by a number of routes (Floyd and Stokinger, 1958). Pretreatment of rats with vitamin E prior to MEKP administration has been shown to reduce the extent of lipid peroxidation in the animal,

with a concurrent decrease in toxicity (Litov *et al.*, 1981). The protective effect of oral vitamin E against lipid peroxidation has been measured in MEKP-treated animals by the reduction in exhaled pentane, which is formed from the decomposition of ω6-unsaturated fatty acid hydroperoxides (Litov *et al.*, 1981; Herschberger and Tappel, 1982). The plasma vitamin E and liver glutathione (GSH) levels of rats were significantly decreased following treatment with MEKP; liver vitamin E levels were less affected. Treatment with dimethyl phthalate alone did not significantly affect vitamin E levels (Warren and Reed, 1991). Other antioxidants, such as selenium, vitamin C, and methionine (alone or in combination), were less effective in reducing lipid peroxidation than vitamin E (Litov *et al.*, 1981).

There are conflicting reports regarding the effects of vitamin E on MEKP-induced damage in the brain of rats. Summerfield and Tappel (1984) showed a protective effect of dietary vitamin E against DNA crosslinking and protein-DNA crosslinking produced by MEKP. Chaudiere *et al.* (1988) found no differences in malonaldehyde levels in the brain of neonatal rats maintained on vitamin E-deficient or sufficient diets and treated with MEKP by intraperitoneal injection. The only difference seen was a small decrease in GSH-reductase activity in the brain of vitamin E-supplemented rats.

MEKP is a substrate for and irreversibly inhibits the activity of microsomal NADH- and NADPH-peroxidase in rats. It also binds to cytochrome P_{450} and inhibits the activity of tetramethylphenylenediamine peroxidase and aminopyrine demethylase (Ando and Tappel, 1985a,b). Administration of vitamin E prior to MEKP injection provided protection against the inhibition of cytochrome P_{450}, NADH- and NADPH-peroxidase, and aminopyrine demethylase activities, but did not affect the inhibition of tetramethylphenylenediamine peroxidase activity (Ando and Tappel, 1985a,b). The induction of these enzymes by phenobarbital prior to treatment with MEKP decreased the level of inhibition by MEKP (Ando and Tappel, 1985a,b). The inhibition of NADH- and NADPH-peroxidase activities was competitive at low concentrations of MEKP. The V_{max} values of these enzymes for MEKP were 33 and 10 nmol NADPH oxidized/min/mg protein, respectively. The corresponding K_m values were 0.022 and 0.012 mM, respectively (Ando and Tappel, 1985b). The effects of MEKP on these microsomal enzymes increased as a function of incubation time and were believed to have been a consequence of the lipid-peroxidizing

ability of MEKP. This was supported by the demonstration, *in vitro*, that MEKP generated more lipid peroxidation products in microsomes from vitamin E-deficient rats than in microsomes from vitamin E-supplemented rats (Ando and Tappel, 1985a).

Glutathione-S-transferase and GSH-peroxidase activities were not affected by MEKP, either in the presence or absence of supplemental vitamin E (Condell and Tappel, 1983; Ando and Tappel, 1985a,b). GSH-peroxidase, which contains selenocysteine at its active site, is more resistant to peroxidative damage than are the sulfhydryl-containing enzymes (Condell and Tappel, 1983).

REPRODUCTIVE TOXICITY

Korhonen *et al.* (1983, 1984) reported that MEKP, administered into the air chamber, was toxic to 3-day chicken embryos, as indicated by increased incidences of dead and malformed embryos. The median effective dose (ED_{50}) was 0.19 µmole MEKP/egg.

TUMORIGENICITY

MEKP (50% in dibutyl phthalate), applied in acetone twice weekly at 10 µg/mouse, showed weak tumor-promoting activity on the skin of male and female hairless mice irradiated with UVB. Dibutyl phthalate without MEKP had no effect. The tumor-promoting activity of MEKP was enhanced by topical treatment with diethyl maleate, which depletes intracellular glutathione, suggesting that lipid peroxidation may play a role in tumor promotion (Logani *et al.*, 1984).

Kotin and Falk (1963) reported that MEKP induced malignant lymphomas in C57B1 mice. The treatment route and regimen, and the corresponding spontaneous tumor incidences, were not reported.

GENETIC TOXICITY

MEKP (1 to 333 µg/plate) dissolved in dimethyl sulfoxide was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98 when tested in a preincubation protocol with and without induced rat and hamster liver S9 (Mortelmans *et al.*, 1986). Low-level mutagenic responses in both plate and preincubation test protocols were shown

in *S. typhimurium* strain TA102, which is reported to be sensitive to oxidative mutagens (Levin *et al.*, 1984). The authors did not indicate in this report whether liver S9 was required. It was reported in an abstract, without supporting data, that MEKP in dimethyl phthalate induced a "slight increase" in sister chromatid exchanges in Chinese hamster ovary cells with and without S9 (Jarventaus *et al.*, 1984). The toxicity of MEKP to the cells was reduced 2- to 3-fold in the presence of S9.

In rats injected intraperitoneally with MEKP, DNA interstrand crosslinks and DNA-protein crosslinks were induced in the brain. Pretreatment of the rats with vitamin E reduced the numbers of both types of crosslinks, presumably by acting as a scavenger of free radicals produced by MEKP (Summerfield and Tappel, 1984).

Study Rationale and Design

MEKP was nominated by the National Cancer Institute (NCI) for toxicity and carcinogenicity testing because of a lack of knowledge of its carcinogenic potential and because of its potential for human exposure. Topical application was chosen as the route of administration for 2-week and 13-week toxicity studies in F344/N rats and B6C3F_1 mice because this is the route by which workers are typically exposed. The studies performed included reproductive system and histopathologic evaluations. MEKP was also evaluated for mutagenicity in *S. typhimurium* and in mouse lymphoma L5178Y cells, for induction of sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells, and for induction of micronuclei in red blood cells in mice in the 13-week studies.

MATERIALS AND METHODS

Procurement and Characterization of Methyl Ethyl Ketone Peroxide and Dimethyl Phthalate

Methyl ethyl ketone peroxide (MEKP; CAS Number 1338-23-4), was obtained in 1 lot (Lot 124-423G) from Witco Chemical Corporation (Richmond, CA) as a 45% w/w solution in dimethyl phthalate (DMP; CAS Number 131-11-3). The same lot of MEKP was used for the 2-week and 13-week studies. Two lots of the DMP vehicle were also obtained from Witco; Lot 124-423G was used in the 2-week studies, and Lots 124-423G and 15334-FMD were used in the 13-week studies.

Identity, purity, and stability analyses were conducted at Midwest Research Institute (Kansas City, MO). The study chemical, a clear, colorless, viscous liquid, was identified as methyl ethyl ketone peroxide in DMP by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The spectra were consistent with a mixture of MEKP and DMP and with available literature references for DMP. Cumulative analytical data, based on spectroscopy, functional group titration, and thin-layer chromatography, indicated a purity of approximately 45% MEKP. Quantitation based on percent of total integration in NMR spectroscopy indicated 44.51% MEKP; UV/visible spectroscopy based on the absorbance at 275 nm indicated the presence of 55% DMP; and iodometric titration of peroxide functional groups indicated a purity of 49.8 ± 0.5% MEKP, equivalent to 9.05 ± 0.09% active oxygen.

Stability studies indicated that 45% MEKP in DMP was stable after 3 hours of exposure to air and normal room lighting. The material was stored in its original plastic containers in the dark at approximately 5°C. At the study laboratory, subsequent chemical reanalyses by iodometric titration and nuclear magnetic resonance spectroscopy revealed consistent purity levels for the bulk chemical relative to the reference standard during these studies.

Identity, purity, and stability analyses were also conducted on both lots of DMP used in these studies. Cumulative analytical data based on spectroscopy, elemental analyses, Karl

Fischer water analysis, functional group titration, thin layer chromatography, and gas liquid chromatography, indicated a purity of at least 98%. Periodic reanalysis of DMP during the studies revealed no degradation. The DMP vehicle was stored at 5°C.

Dose Formulations

The test material was a 45% solution (w/w) of MEKP in DMP. It was applied without further dilution at different volumes in the 2-week studies. In the 13-week studies, dose formulations were prepared by diluting the bulk material with DMP to achieve the desired dose levels. Dose formulations were stored in the dark at 5°C. Results of analyses of dose formulations by functional group titration, before and after administration to animals in the 13-week studies, were within 10% of theoretical values.

Toxicity Study Designs

Male and female F344/N rats and B6C3F$_1$ mice used in these studies were produced under strict barrier conditions at Taconic Farms, Germantown, NY. Rats and mice were shipped to the study laboratory (Hazleton Laboratories America, Inc.) at approximately 3 weeks of age (13-week study in mice) to 4 weeks of age (2-week studies and 13-week study in rats), quarantined for 13 to 18 days, and then placed on study at about 6 weeks of age. Blood samples were collected from 5 untreated control animals per sex and species at the start and termination of the 13-week studies. The sera were analyzed for viral antibody titers; data from 5 viral screenings performed in rats and 12 screenings performed in mice (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b) showed no positive antibody titers. Additional details concerning study design and performance are described in Table 1.

Rats and mice were housed individually for all studies. Animal room temperatures ranged from 68°F to 75°F; relative humidity ranged from 31% to 72% with 12 to more than 20 fresh air changes per hour. Fluorescent light was provided for 12 hours per day. Feed and water were available *ad libitum*.

In the 2-week study in rats, doses of 0 (untreated and DMP controls), 50.6, 101.3, 202.5, 405, or 810 mg MEKP/kg body weight were applied topically to the clipped dorsal skin.

In the 2-week study in mice, MEKP in DMP (45:55 w/w) was administered topically to the clipped dorsal skin at doses of 0 (untreated and DMP controls), 112.5, 225, 450, 900, or 1800 mg/kg body weight. For both 2-week studies, groups of 5 animals per sex per species were weighed individually and dosed on the basis of group mean body weights. MEKP was applied at different dose volumes to attain the different dose levels. Dose volumes were adjusted weekly based on changes in group mean body weights. The vehicle control group was treated with DMP alone at a volume equivalent to 0.4 times the volume of MEKP applied to the highest dose group so as to be equivalent to the DMP concentration in MEKP. In addition, an untreated control group was clipped and handled in the same manner as the DMP control and MEKP groups, but was not dosed. The test article was administered once a day for 5 days per week for 2 weeks, plus 2 consecutive dose days before terminal sacrifice.

Doses for the 13-week studies were based on the results of the 2-week studies. In the 13-week studies, MEKP in DMP (45:55 w/w) was diluted with DMP to achieve 0.3, 1.0, 3.0, 10.0, and 30.0% (w/w) solutions. Groups of 10 animals per sex per species were dosed at a volume of 0.3 mL per rat and 0.1 mL per mouse. MEKP was administered topically to the clipped dorsal skin of rats at doses of 0 (untreated and DMP controls), 1.07, 3.57, 10.7, 35.7, or 107 mg/animal. MEKP was also administered topically to the clipped dorsal skin of mice at doses of 0 (untreated and DMP controls), 0.357, 1.19, 3.57, 11.9, or 35.7 mg/animal. DMP controls were dosed with 0.3 mL DMP per rat and 0.1 mL DMP per mouse; untreated controls were clipped and handled, but were not dosed. The test article was administered once a day for 5 days per week, except holidays, for a total of 13 weeks, plus 2 consecutive dose days before terminal sacrifice.

Complete necropsies were performed on all animals. The liver, thymus, right kidney, right testis, heart, brain, lungs, and spleen (13-week studies only) from animals killed at the end of the studies were weighed prior to fixation. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Complete histopathologic examinations of protocol-required tissues were performed on all control animals, all animals that died early, all animals in the highest dose group with at least 60% survivors at the time of sacrifice, plus all animals

in the higher dose groups inclusive of early deaths and survivors. Selected tissues and gross lesions were examined microscopically in animals from lower dose groups until a no-effect level was determined. Because a no-effect level was not seen following treatment with MEKP, tissues from all dose groups were examined. Selected tissues examined, and those required by the protocol to be examined, are listed in Table 1.

Upon completion of the laboratory pathologist's histologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. The results were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

Vaginal cytology and sperm morphology evaluations were performed on rats dosed with the DMP vehicle or with 1.07, 3.57, or 10.7 mg MEKP/animal and on mice dosed with the DMP vehicle or with 0.357, 1.19, or 3.57 mg MEKP/animal during the 13-week studies. Methods were those described by Morrissey *et al.* (1988). Briefly, for 7 days prior to sacrifice, the vaginal vaults of 10 females of each species and dose group were lavaged, and the aspirated lavage fluid and cells were stained with Toluidine Blue. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (*i.e.*, diestrus, proestrus, estrus, or metestrus).

Sperm motility was evaluated at necropsy in the following manner. The right testis and epididymis were weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Tyrode's buffer (mice) or egg yolk (rats) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides and the numbers of motile and nonmotile spermatozoa were counted for 5 fields per slide.

Following completion of sperm motility estimates, each right cauda epididymis was placed in buffered 0.9% saline solution. Cauda were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemacytometer.

Peripheral blood smears for determination of micronuclei were prepared from all untreated, DMP control, and MEKP-treated mice, although slides prepared from mice in the 11.9 and 35.7 mg/animal groups were not evaluated. Details of this procedure are described in the following section.

Genetic Toxicity Studies

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Mutagenicity studies of MEKP in *Salmonella typhimurium* were conducted as reported by Mortelmans *et al.* (1986). MEKP in DMP (45:55) was sent to the laboratory as a coded aliquot. It was diluted in dimethyl sulfoxide and incubated for 20 minutes at 37°C with the *S. typhimurium* tester strains (TA98, TA100, TA1535, or TA1537) either in buffer or S9 mix (9000 × g liver homogenate supernatant from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver, and cofactors). Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37°C. Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least 5 doses of MEKP; the high dose was limited by toxicity. All assays were repeated.

MOUSE LYMPHOMA MUTAGENICITY TEST PROTOCOL

The experimental protocol is presented in detail by Myhr *et al.* (1985). MEKP in DMP (45:55) was supplied as a coded aliquot. The highest dose used was determined by toxicity. Mouse lymphoma L5178Y cells were maintained at 37°C as suspension cultures in supplemented Fischer's medium; normal cycling time was about 10 hours. To reduce the number of spontaneously occurring trifluorothymidine-resistant cells, subcultures were exposed to medium containing THMG (thymidine, hypoxanthine, methotrexate, and

glycine) for 1 day, to THG for 1 day, and to normal medium for 3 to 5 days. For cloning, horse serum content was increased and Noble agar was added.

All treatment levels and controls within an experiment were replicated. Treated cultures contained 6 x 10^6 cells in 10 mL of medium. Incubation with MEKP (diluted in ethanol) was for 4 hours, then the medium plus MEKP was removed; the cells were resuspended in fresh medium and incubated for an additional 2 days to allow expression of the mutant phenotype. Log phase growth was maintained. After the expression period, 3 x 10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine (TFT) for selection of TFT-resistant ($TK^{-/-}$) cells. In addition, 600 cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37°C in 5% CO_2 for 10 to 12 days.

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al.* (1987). MEKP in DMP (45:55) was supplied as a coded aliquot. It was diluted in dimethyl sulfoxide and was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and in the absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls, and of at least 3 doses of MEKP; the high dose was limited by toxicity.

In the SCE test without S9, CHO cells were incubated for 26 hours with MEKP in McCoy's 5A medium supplemented with fetal bovine serum, *l*-glutamine, and antibiotics. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing MEKP was removed and replaced with fresh medium plus BrdU and Colcemid. Incubation was continued for an additional 2 to 3 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with MEKP, serum-free medium, and S9 mix for 2 hours. The medium was removed and replaced with medium containing BrdU and no MEKP, and incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9.

In the chromosome aberration (Abs) test without S9, cells were incubated in McCoy's 5A medium with MEKP for 10 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with MEKP and S9 mix for 2 hours, after which the treatment medium was removed and the cells incubated for 8 to 10 hours in fresh medium; Colcemid was present for the final 2 hours of this period. Cells were harvested in the same manner as for the treatment without S9.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. For the SCE test, 50 second-division metaphase cells were scored for frequency of SCEs per cell from each dose level; 100 first-division metaphase cells were scored at each dose level for the Abs test. Classes of aberrations recorded included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented in MacGregor *et al.* (1983). At the conclusion of the 13-week toxicity study, blood was obtained by cardiac puncture of anesthetized mice, and smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with a chromatin-specific fluorescent dye mixture of Hoechst 33258/pyronin Y (MacGregor *et al.*, 1983) and were coded. Slides were scanned using a semi-automated image analysis system to determine the frequency of micronuclei in 10,000 normochromatic erythrocytes (NCEs) and 2000 polychromatic erythrocytes (PCEs) for each of 10 males and 10 females per dose group. The criteria of Schmid (1976) were used in defining micronuclei, with the additional requirement that micronuclei exhibit the characteristic fluorescent emissions of DNA (blue with 360 nm, and orange with 540 nm UV illumination). The percentage of PCEs among the total erythrocyte population was also determined.

TABLE 1 Experimental Design and Materials and Methods in the 2-Week and 13-Week Dermal Studies of Methyl Ethyl Ketone Peroxide

EXPERIMENTAL DESIGN	
Study Laboratory	Hazleton Laboratories America, Inc , Rockville, MD
Size of Study Groups	2-Week Studies 5 males and 5 females of each species per dose group 13-Week Studies 10 males and 10 females of each species per dose group
Route of Administration	Dosing solutions were applied topically to the clipped dorsal skin
Dose Volume	2-Week Studies Dose volumes were adjusted weekly based on changes in group mean body weights 13-Week Studies Rats 0 3 mL/animal Mice 0 1 mL/animal
Concentration of Dose Formulations	2-Week Studies Rats and Mice 0 (untreated and DMP controls) or 45% (w/w) MEKP in DMP 13-Week Studies Rats and Mice 0 (untreated and DMP controls), 0 3, 1 0, 3 0, 10, or 30% (w/w) MEKP in DMP
Doses/Duration of Dosing	2-Week Studies Rats 0 (untreated and DMP), 50 6, 101 3, 202 5, 405, or 810 mg MEKP/kg, 5 days per week for 2 weeks, plus 2 consecutive dose days before terminal sacrifice Mice 0 (untreated and DMP), 112 5, 225, 450, 900, or 1800 mg MEKP/kg, 5 days per week for 2 weeks, plus 2 consecutive dose days before terminal sacrifice 13-Week Studies Rats 0 (untreated and DMP), 1 07, 3 57, 10 7, 35 7, or 107 mg MEKP/animal, 5 days per week, except for holidays, for 13 weeks plus 2 consecutive dose days before terminal sacrifice Mice 0 (untreated and DMP), 0 357, 1 19, 3 57, 11 9, or 35 7 mg MEKP/animal, 5 days per week, except for holidays, for 13 weeks plus 2 consecutive dose days before terminal sacrifice
Date of First Dose	2-Week Studies Rats, 13 October 1986 Mice, 13 October 1986 13-Week Studies Rats, 6 July 1987 Mice, 20 July 1987
Date of Last Dose	2-Week Studies Rats, 29 October 1986 Mice, 28 October 1986 13-Week Studies Rats, 7 October 1987 Mice, 20-21 October 1987

TABLE 1 Experimental Design and Materials and Methods in the 2-Week and 13-Week Dermal Studies of Methyl Ethyl Ketone Peroxide (continued)

Necropsy Dates	2-Week Studies Rats, 30 October 1986 Mice, 29 October 1986 13-Week Studies Rats, 7-8 October 1987 Mice, 21-22 October 1987
Type and Frequency of Observation	2-Week Studies Observed twice daily for mortality/morbidity Clinical signs of toxicity were recorded daily Animals were weighed individually on Day 1, then once during dosing, and at necropsy 13-Week Studies Observed twice daily for mortality/morbidity Clinical observations were recorded weekly Individual body weights were recorded on Day 1, weekly thereafter, and at necropsy
Necropsy and Histologic Examinations	A complete necropsy was performed on all animals The protocol required that tissues be examined microscopically in all control animals, all animals that died early, all animals in the highest dose group with at least 60% survivors, and all animals in higher dose groups inclusive of early deaths and survivors These tissues included adrenal glands, bone (femur 13 week studies or sternum 2 week studies) with marrow, brain (3 sections), esophagus, gallbladder (mice), gross lesions, heart/aorta, intestines (large cecum, colon, rectum, small duodenum, jejunum, ileum), kidneys, liver, lung/mainstem bronchi, lymph nodes (mandibular, mesenteric), mammary gland, nasal cavity and turbinates (3 sections), ovaries, pancreas, parathyroid glands, pituitary gland, preputial or clitoral glands, prostate gland, salivary glands, seminal vesicles, skin (application site and untreated inguinal), spleen, stomach (forestomach and glandular stomach), testes (with epididymis), thymus, thyroid gland, trachea, urinary bladder, and uterus Selected organs examined in all rats and mice were skin (application site), spleen, and bone marrow
Supplemental Evaluations	Sperm Morphology and Vaginal Cytology Sperm morphology and vaginal cytology evaluations were performed at the end of the 13-week studies Rats dosed with the DMP vehicle, 1 07, 3 57, or 10 7 mg MEKP/animal and mice dosed with the DMP vehicle, 0 357, 1 19, or 3 57 mg MEKP/animal were evaluated Males were evaluated for necropsy body and reproductive tissue weights and spermatozoal data Females were evaluated for necropsy body weight, estrous cycle length, and the percent of cycle spent in the various stages Peripheral Blood Micronucleus Assay Peripheral blood samples were taken from all mice at the time of necropsy for evaluation of micronuclei in normochromatic and polychromatic erythrocytes
ANIMALS AND ANIMAL MAINTENANCE	
Strain and Species	F344/N Rats B6C3F$_1$ Mice
Animal Source	Taconic Farms, Germantown, NY

TABLE 1 Experimental Design and Materials and Methods In the 2-Week and 13-Week Dermal Studies of Methyl Ethyl Ketone Peroxide (continued)

Time Held Before Study	2-Week Studies Rats, 13 days, Mice, 14 days 13 Week Studies Rats, 13 days, Mice, 18 days
Age When Study Began	2-Week Studies Rats, 6 weeks, Mice, 6 weeks 13-Week Studies Rats, 6 weeks, Mice, 6 weeks
Age When Killed	2-Week Studies Rats, 8 weeks, Mice, 8 weeks 13 Week Studies Rats, 19 weeks, Mice, 19 weeks
Method of Animal Distribution	Animals were weighed and randomized using a computer program
Diet	NIH-07 Open Formula Pellets (Zeigler Brothers, Inc , Gardners, PA) and tap water available *ad libitum*
Animal Room Environment	Rats and mice were housed individually for all studies Temperature ranged from 68°F to 75°F, relative humidity ranged from 31% to 72% with 12 to more than 20 air changes per hour Fluorescent light was provided for 12 hours per day

Statistical Methods

ANALYSIS OF CONTINUOUS VARIABLES

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972) and Dunnett (1955). Data that typically have skewed distributions were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) or Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunnett, Dunn). If the P-value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test.

The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value. The extreme values chosen by the statistical test were subject to approval by NTP personnel. In addition, values indicated

by the laboratory report as being inadequate due to technical problems were eliminated from the analysis.

ANALYSIS OF VAGINAL CYTOLOGY DATA

Because the data are proportions (the proportion of the observation period that an animal was in a given estrous state), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across dose levels.

ANALYSIS OF MUTAGENICITY IN *SALMONELLA TYPHIMURIUM*

A positive response in the *Salmonella typhimurium* test was defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any 1 strain/activation combination. An equivocal response was defined as an increase in revertants that was not dose related, not reproducible, or not of sufficient magnitude to support a determination of mutagenicity. A negative response was obtained when no increase in revertant colonies was observed following chemical treatment. There was no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

ANALYSIS OF MOUSE LYMPHOMA MUTAGENICITY DATA

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented in Caspary *et al.* (1988). Data were evaluated statistically for both trend and peak responses. Both responses had to be significant ($P \leq 0.05$) for a chemical to be considered capable of inducing TFT-resistance. A single significant response led to a "questionable" conclusion, and the absence of both a trend and a peak response resulted in a "negative" call.

ANALYSIS OF CHO CELL CYTOGENETICS DATA

For the SCE data, statistical analyses were conducted on the slopes of the dose-response curves (Galloway *et al.*, 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability

of this level of difference occurring by chance at 1 dose point is less than 0.01; the probability for such a chance occurrence at 2 dose points is less than 0.001. If only 1 dose was increased by at least 20% over the solvent control, it was considered weak evidence for a positive response; increases in at least 2 doses resulted in a determination of positive. A statistically significant trend ($P \leq 0.05$) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal aberration data are presented as percentage of cells with aberrations; both the dose-response curve and individual dose points were statistically analyzed (Galloway *et al.*, 1987). For a single trial, a statistically significant ($P \leq 0.05$) difference for 1 dose point and a significant trend ($P \leq 0.015$) were considered weak evidence for a positive response; significant differences for 2 or more doses indicated the trial was positive.

ANALYSIS OF MOUSE PERIPHERAL BLOOD MICRONUCLEUS DATA

Log transformation of the normochromatic erythrocyte (NCE) data, and testing for normality by the Shapiro-Wilk test, and for heterogeneity of variance by Cochran's test, were performed before statistical analyses. The frequency of micronucleated cells among NCEs was analyzed by an analysis of variance using the SAS GLM procedure. The NCE data for each dose group were compared with data from the concurrent DMP control using Student's *t*-test. The frequency of micronucleated cells among polychromatic erythrocytes (PCEs) was analyzed by the Cochran-Armitage trend test, and individual dose groups were compared to the concurrent DMP control by Kastenbaum-Bowman's (1970) binomial test. The percentage PCE among total erythrocytes was analyzed by an analysis of variance on ranks (classed by sex) and individual dose groups were compared with the concurrent DMP control using a *t*-test on ranks.

Quality Assurance

The animal studies of MEKP were performed in compliance with the United States FDA Good Laboratory Practices regulations (21 CFR 58). The Quality Assurance Unit of Hazleton Laboratories America, Inc. performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies. The operations of the Quality Assurance Unit were monitored by the NTP.

RESULTS

2-Week Dermal Study in F344/N Rats

All rats treated with methyl ethyl ketone peroxide (MEKP) for 2 weeks survived to the end of the study (Table 2). There was a dose-dependent decrease in the mean body weight gain compared to the dimethyl phthalate (DMP) control group for all MEKP-treated male rats and for female rats in the 2 highest (405 and 810 mg/kg) dose groups. A dose-related decrease in mean final body weights also occurred in rats given MEKP.

TABLE 2 Survival and Weight Gain of F344/N Rats in the 2-Week Dermal Study of Methyl Ethyl Ketone Peroxide

Dose (mg/kg)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to DMP Controls (%)[3]
		Initial	Final	Change[2]	
MALE					
Untreated	5/5	125	200	75	102
DMP Control	5/5	127	196	69	—
50 6	5/5	128	187	59	96
101 3	5/5	124	176	53	90
202 5	5/5	136	184	48	94
405	5/5	129	177	49	90
810	5/5	134	162	28	83
FEMALE					
Untreated	5/5	112	145	33	99
DMP Control	5/5	115	147	32	—
50 6	5/5	112	144	32	98
101 3	5/5	114	141	27	96
202 5	5/5	112	141	30	96
405	5/5	114	136	22	93
810	5/5	113	135	21	92

[1] Number surviving at 18 days/number of animals per dose group
[2] Mean weight change of the animals in each dose group
[3] (Dosed group mean/DMP control group mean) × 100

Changes in organ weights that were considered to be possibly biologically significant were limited to decreases in absolute and/or relative thymus weights in males receiving 101.3 to 810 mg MEKP/kg and in females receiving 202.5 to 810 mg MEKP/kg, and a trend toward mild increases in relative liver weights in MEKP-treated male and female rats when

compared to the DMP controls (statistically significant in females receiving 202.5 to 810 mg/kg) (Table 3).

TABLE 3 Liver and Thymus Weights of F344/N Rats Administered Methyl Ethyl Ketone Peroxide Topically for 2 Weeks[1]

			MEKP (mg/kg)				
	Untreated	DMP Control	50.6	101.3	202.5	405	810
MALE							
Necropsy body weight	211	206	194	185	189	184*	165**
Liver weight	11 344	11 234	10 632	10 384	11 050	10 642	9 880
Relative liver weight	53 83	54 37	55 16	56 00	58 33	57 87	59 78
Thymus weight	0 468	0 479	0 401	0 348*[2]	0 374*	0 358**	0 265**
Relative thymus weight	2 22	2 33	2 07	1 85[2]	1 98	1 94	1 61**
FEMALE							
Necropsy body weight	150	151	146	145	141*	138**	135**
Liver weight	6 690	7 260	7 608	7 236	7 622	7 514	8 082
Relative liver weight	44 57*	48 02	52 18	49 92	54 14**	54 50**	59 70**
Thymus weight	0 350	0 370	0 361	0 345	0 291*	0 305*	0 273**
Relative thymus weight	2 33	2 44	2 46	2 39	2 06	2 21	2 03*

[1] Organ weights and body weights are given in grams, relative organ weights (organ weight-to-body-weight ratios) are given as mg organ weight/g body weight, n=5 except where noted, DMP = dimethyl phthalate
[2] n=4
* Significantly different (P≤0 05) from the DMP control group by Williams' test
** Significantly different (P≤0 01) from the DMP control group by Williams' test

At necropsy, gross lesions associated with compound administration were observed at the site of application of MEKP to the skin. The skin was thickened and indurated in all rats in all MEKP-treated groups. A scab-like crust was also present over the application site in some animals in the 405 and 810 mg/kg groups. An enlarged spleen was noted in 1 male and in 1 female rat receiving 810 mg MEKP/kg.

Gross lesions at the application site were correlated with MEKP-related dermal/epidermal necrosis and associated inflammatory reactions. The typical histologic presentation at all MEKP dose levels was extensive coagulative necrosis of the epidermis and dermis as well as the adnexa. The necrotic skin generally formed a superficial coagulum; the viable tissue underneath exhibited variable degrees of regeneration and inflammation.

Regeneration was characterized by incomplete to complete bridging of denuded areas by epithelium, as well as by the consistent presence of epidermal hyperplasia (acanthosis) at the margins of ulcerated areas. Inflammation was usually marked and consisted primarily of neutrophilic and serous exudate superficially, and of fibrovascular proliferation ("granulation tissue") with mixed leukocyte infiltration in the deep dermis and subcutis. The severity of the necro-inflammatory lesions was moderate to marked at all MEKP dose levels, with a tendency for somewhat more extensive involvement at the higher dose levels.

MEKP-related changes were also present in the spleen. Increased hematopoietic cell proliferation relative to DMP and untreated controls and congestion of the red pulp were observed in all MEKP-treated rats. Increased hemosiderin deposition and focal infiltration of neutrophils and histiocytic cells into the splenic capsule were also observed in the spleen, primarily at the higher dose levels.

13-Week Dermal Study in F344/N Rats

MEKP was highly toxic when applied to the skin of rats, 5 days per week, for up to 13 weeks. Three females in the high-dose (107 mg/animal) group died or were killed moribund during Week 1 of the study. All animals in the 35.7 mg/animal group and all remaining animals in the 107 mg/animal group were killed during Week 8 due to the severity of the skin lesions at the site of MEKP application (Table 4). The mean final body weight and mean body weight gain for untreated controls were greater than the corresponding values for DMP controls. In addition, mean final body weights and mean body weight gains decreased with dose for rats treated with MEKP (Figure 1). Body and organ weight analyses were not performed for rats in the 35.7 and 107 mg/animal groups due to the early death of all animals in these groups.

TABLE 4 Survival and Weight Gain of F344/N Rats in the 13-Week Dermal Study of Methyl Ethyl Ketone Peroxide

Dose (mg/animal)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to DMP Controls (%)[4]
		Initial[2]	Final	Change[3]	
MALE					
Untreated	10/10	127	365	238	106
DMP control	10/10	121	344	223	
1 07	10/10	134	362	228	105
3 57	10/10	127	351	224	102
10 7	10/10	128	300	172	87
35 7	0/10[5]	126	—	—	—
107	0/10[5]	128	—	—	—
FEMALE					
Untreated	10/10	107	215	109	103
DMP control	10/10	109	210	101	
1 07	10/10	106	203	98	97
3 57	10/10	108	206	98	98
10 7	10/10	108	199	91	95
35 7	0/10[5]	109	—	—	—
107	0/10[6]	107	—	—	—

[1] Number surviving at 13 weeks/number of animals per dose group For groups with no survivors, no final mean body weights or body weight changes are given
[2] Body weights were measured at Day 1 Subsequent calculations are based on animals surviving to the end of the study
[3] Mean weight change of the survivors
[4] (Dosed group mean/DMP control group mean) × 100
[5] Week of death 8 (killed due to severity of skin lesions)
[6] Week of death 1 (1 killed in a moribund condition, 2 early deaths), 8 (7 killed due to severity of skin lesions)

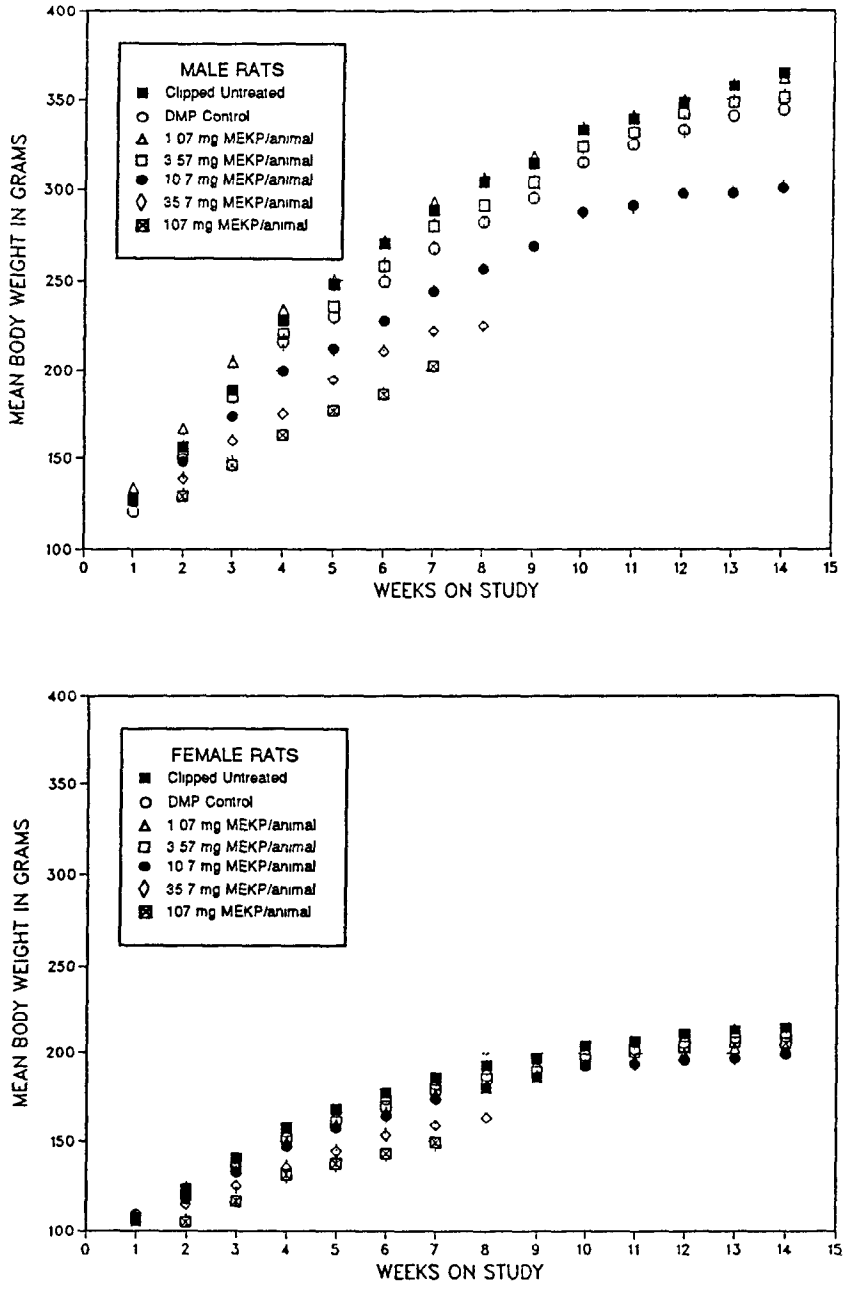

FIGURE 1 Body Weights of F344/N Rats Exposed to Methyl Ethyl Ketone Peroxide by Topical Administration for 13 Weeks

There were statistically significant increases in the relative weights of several organs, primarily in males in the 10.7 mg/animal group (Appendix A). These were considered secondary to the lower body weight gains in these animals and not reflective of a primary chemical effect. There were no changes in absolute or relative organ weights that were considered treatment related. However, the relative liver weights of the DMP control female rats showed greater variability, and the mean was somewhat greater than those of the untreated controls or of the MEKP-treated groups.

Necropsy observations showed thickened, crusty skin at the site of MEKP application, particularly in the 35.7 and 107 mg dose group animals that were sacrificed early. Additional observations in many of the early-death rats receiving 107 mg/animal were "scar tissue" at the application site and enlarged spleens. In those animals that survived for 13 weeks, significant necropsy observations were limited to thick, crusty skin at the application site in several animals from the 10.7 mg/animal group.

Microscopically, MEKP treatment was associated with a spectrum of necrotic, inflammatory, and regenerative skin lesions at the application site; the severity of the lesions increased progressively with dose. Dermal and epidermal necrosis was the primary lesion at dose levels of 10.7 mg/animal and greater. Necrosis varied in severity from diffuse involvement of both dermis and epidermis at higher concentrations to focal ulcerations of the epidermis, which occurred more frequently in the 10.7 mg/animal group. An associated inflammatory reaction consisted of both surface exudation and dermal inflammation. Typically, a coagulum of surface exudate containing serous fluid and neutrophils was seen overlying denuded or regenerative areas, with necrotic skin tissue admixed in more severe cases. The dermal reaction was subjacent to ulcerated, sloughed, or regenerated epidermis and was collectively termed "chronic-active" to be inclusive of varied patterns, including primarily neutrophilic, fibrovascular ("granulation tissue"), granulomatous, or fibrotic reactions. Epidermal hyperplasia (acanthosis) and hyperkeratosis were consistently present at the margins of ulcerated areas in the higher dose groups (10.7 to 107 mg/animal). In the 1.07 and 3.57 mg/animal groups, acanthosis and/or hyperkeratosis were the only skin lesions evident at the application site; these lesions were seen in all animals in these dose groups. Minimal acanthosis and hyperkeratosis were seen in some untreated and DMP control rats.

Treatment-related microscopic changes were also found in the spleen and femoral bone marrow, primarily in the 35.7 and 107 mg/animal groups. In the spleen, there were minimal to moderate increases in hematopoietic cell proliferation in the red pulp. In the bone marrow, hematopoietic cell proliferation was also observed, but involved primarily myeloid cells and was termed "myeloid hyperplasia." At the highest dose (107 mg/animal), an additional finding of necrosis of respiratory epithelial cells in the nasal cavity was also attributed to MEKP treatment, possibly resulting from preening activity with aspiration of the compound. This lesion was characterized by individual cell necrosis in the dorsal and middle meatus of the anterior and middle nasal sections and was frequently accompanied by intra-epithelial and sub-epithelial inflammation.

In addition to skin lesions at the application site, histopathologic changes seen in the 3 high-dose females that died or were killed during the first week of the study included acute centrilobular degeneration and necrosis in the liver, acute tubule necrosis in the kidney, lymphoid necrosis in the spleen and thymus, and hypertrophy of the adrenal cortex. These lesions were considered to be secondary to agonal hypoxia and/or stress. Table 5 summarizes the incidence and severity of selected lesions in rats from the 13-week dermal study of MEKP.

TABLE 5 Incidence[1] and Severity[2] of Selected Treatment-Related Lesions in F344/N Rats Administered Methyl Ethyl Ketone Peroxide Topically for 13 Weeks

			MEKP (mg/animal)				
	Untreated	DMP Control	1.07	3.57	10.7	35.7	107
MALE							
Skin (SOA)[3]							
Ulcer/necrosis	0	0	0	0	7 (1.4)	10 (3.7)	10 (4.2)
Chronic-active inflammation	0	0	0	0	10 (2.0)	10 (3.5)	10 (3.9)
Acanthosis	3 (1.3)	2 (1.0)	10 (1.0)	10 (1.4)	10 (2.9)	10 (3.2)	10 (3.0)
Hyperkeratosis	3 (1.0)	0	10 (1.3)	10 (1.1)	10 (1.9)	10 (2.2)	10 (2.1)
Spleen							
Hematopoietic cell proliferation	0	0	0	0	0	4 (1.3)	5 (2.8)
Bone Marrow							
Myeloid hyperplasia	0	0	0	0	1 (1.0)	8 (1.9)	9 (2.7)
FEMALE							
Skin (SOA)							
Ulcer/necrosis	0	0	0	0	6 (1.3)	9 (3.6)	10 (4.6)
Chronic-active inflammation	0	0	0	0	10 (1.8)	10 (3.5)	7 (4.6)
Acanthosis	2 (1.0)	0	0	9 (1.2)	10 (2.9)	10 (3.3)	7 (3.1)
Hyperkeratosis	0	0	10 (1.0)	10 (1.5)	10 (1.9)	10 (2.0)	6 (2.0)
Spleen							
Hematopoietic cell proliferation	0	0	0	0	1 (1.0)	6 (2.3)	4 (2.5)
Bone Marrow							
Myeloid hyperplasia	0	0	0	0	0	8 (2.4)	10 (3.5)

[1] Incidence is the number of animals with lesions from groups of 10
[2] Average severity score ( ) was based on the number of animals with lesions, 1 = minimal, 2 = slight, 3 = mild, 4 = moderate, 5 = marked
[4] SOA = site of application

Sperm morphology and vaginal cytology evaluations were performed on rats in the DMP control, 1.07, 3.57, and 10.7 mg MEKP/animal groups (Appendix B). None of the parameters examined were affected by MEKP treatment when compared to the DMP control (Appendix B).

2-Week Dermal Study in B6C3F$_1$ Mice

MEKP was acutely toxic when applied to the skin of mice 5 days per week for 2 weeks. Three females and 4 males in the high-dose (1800 mg/kg) group died by Day 7 of the study (Table 6). In addition, 1 female and 2 males in the 900 mg/kg group, 1 female and 1 male in the 450 mg/kg group, and 2 males in the 225 mg/kg group died by Day 7; 1 female in the 112.5 mg/kg group died on Day 8. Early deaths were attributed to the severe skin lesions that occurred at the site of MEKP application. Final mean body weights and mean body weight gains of untreated and MEKP-treated mice were not significantly different from those of DMP-treated animals.

TABLE 6 Survival and Weight Gain of B6C3F$_1$ Mice in the 2-Week Dermal Study of Methyl Ethyl Ketone Peroxide

Dose (mg/kg)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[4]
		Initial[2]	Final	Change[3]	
MALE					
Untreated	5/5	23 6	26 3	2 7	100
DMP Control	5/5	23 7	26 3	2 6	—
112 5	5/5	23 6	27 3	3 7	104
225	3/5[5]	23 1	23 2	0 2	88
450	4/5[5]	23 8	26 7	3 0	102
900	3/5[6]	24 6	27 3	2 1	104
1800	1/5[7]	23 2	27 0	2 0	103
FEMALE					
Untreated	5/5	20 0	22 9	2 9	104
DMP Control	5/5	19 9	21 9	2 1	—
112 5	4/5[8]	19 2	22 6	3 3	103
225	5/5	19 2	23 1	3 9	105
450	4/5[5]	19 5	21 9	2 3	100
900	4/5[9]	20 3	22 6	2 3	103
1800	2/5[10]	20 0	23 4	3 1	106

[1] Number surviving at 16 days/number of animals per dose group
[2] Initial group mean body weight Subsequent calculations are based on animals surviving to the end of the study
[3] Mean body weight change of the survivors
[4] (Dosed group mean/DMP control group mean) × 100
[5] Day of death all on Day 7
[6] Day of death 4, 7
[7] Day of death 2, 3, 5, 5
[8] Day of death 8
[9] Day of death 5
[10] Day of death 3, 3, 7

Changes in liver and thymus weights were related to chemical treatment (Table 7). Significant increases in absolute and relative liver weights were noted for male and female mice treated with MEKP. In addition, the absolute and relative liver weights of DMP controls were greater than those of the untreated control group. Both absolute and relative thymus weights were significantly lower than DMP control values for females in the 3 highest dose groups (450 to 1800 mg/kg); males in the 225 and 900 mg/kg dose groups also had lower thymus weights than DMP control males, but the differences were not statistically significant.

TABLE 7 Liver and Thymus Weights of B6C3F$_1$ Mice Administered Methyl Ethyl Ketone Peroxide Topically for 2 Weeks[1]

			MEKP (mg/kg)				
	Untreated	DMP Control	112.5	225	450	900	1800
MALE							
n	5	5	5	3	4	3	1
Necropsy body weight	26 7	26 5	27 0	21 7	24 9	23 0	21 7
Liver weight	1 284	1 376	1 504	1 607*	1 878**	2 233**	2 410
Relative liver weight	48 11*	51 85	55 73	63 09*	68 85**	80 44**	88 93
Thymus weight	0 048	0 049	0 051	0 027[2]	0 054	0 024	0 060
Relative thymus weight	1 80	1 86	1 91	1 01[2]	2 01	0 85	2 21
FEMALE							
n	5	5	4	5	4	4	2
Necropsy body weight	22 7	23 2	20 4	24 2	22 6	22 1	18 9
Liver weight	1 120*	1 268	1 375	1 552	1 590	1 778**	1 710*
Relative liver weight	49 22**	54 57	61 01*	64 20**	67 99**	76 24**	76 32**
Thymus weight	0 079	0 084	0 065	0 068	0 049*	0 053*	0 054*
Relative thymus weight	3 48	3 62	2 87	2 81	2 02**	2 23**	2 41*

[1] Organ weights and body weights are given in grams, relative organ weights (organ-weight-to-body weight ratios) are given as mg organ weight/g body weight

[2] n=2

* Significantly different (P≤0 05) from the DMP control group by Williams' test

** Significantly different (P≤0 01) from the DMP control group by Williams' test

Gross skin lesions at the application site were seen in all MEKP-treated mice at necropsy. The skin was thickened, crusty, hardened, and, in some animals, sloughed. Splenic enlargement was noted in 1 or more animals in most MEKP treatment groups.

Microscopically, MEKP application was associated with necrotic and inflammatory lesions of the skin and underlying tissue at the application site in all dose groups. Coagulative necrosis of the skin was the primary lesion; it was graded as minimal to marked in severity. Superficial exudation of neutrophils and proteinaceous fluid invariably accompanied the necrotic tissue. Proliferation of fibrovascular ("granulation") tissue was variably present and was seen more frequently in mice surviving to the end of the study. Other histologic findings attributed to MEKP treatment, but believed to be secondary to the skin inflammation and necrosis, were minimal to moderate hematopoietic cell proliferation in the spleen at all dose levels and mild to marked myeloid hyperplasia of the sternebral bone marrow, which was most evident in females in the 900 and 1800 mg/kg groups. There were no microscopic findings in the liver to account for the increased liver weights.

Gross and microscopic findings in animals that died early were generally similar to those present in mice that survived to the end of the study. However, lymphoid cell depletion and necrosis in the thymus were additional lesions noted upon microscopic examination of mice that died early.

13-Week Dermal Study in B6C3F$_1$ Mice

MEKP was acutely toxic when applied to the skin of mice 5 days per week for 13 weeks. All animals in the high-dose (35.7 mg/animal) group and 1 female in the 11.9 mg/animal group died during Week 1 of the study; the deaths were attributed to severe skin lesions at the application site. Because of the early onset and severity of the skin lesions, a decision was made to kill the remaining males and females in the 11.9 mg/animal groups. Mean final body weights and body weight gains of animals treated with MEKP were similar to those of the DMP controls; however, untreated control females gained notably more weight than DMP controls during the study (Table 8, Figure 2). Body weights and organ weights were not analyzed for mice in the 11.9 and 35.7 mg/animal groups due to the early death of all animals in these groups.

TABLE 8 Survival and Weight Gain of B6C3F$_1$ Mice in the 13-Week Dermal Study of Methyl Ethyl Ketone Peroxide

Dose (mg/animal)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to DMP Controls (%)[4]
		Initial[2]	Final	Change[3]	
MALE					
Untreated	10/10	24 2	34 6	10 4	103
DMP Control	10/10	24 6	33 6	9 0	
0 357	10/10	24 3	33 2	8 9	99
1 19	10/10	24 6	33 3	8 7	99
3 57	10/10	23 6	32 0	8 3	95
11 9	0/10[5]	24 0	—	—	—
35 7	0/10[6]	24 3	—	—	—
FEMALE					
Untreated	10/10	19 6	29 2	9 6	109
DMP Control	10/10	19 5	26 9	7 4	
0 357	10/10	20 0	28 3	8 3	105
1 19	10/10	19 3	26 9	7 5	100
3 57	10/10	19 6	27 4	7 8	102
11 9	0/10[7]	19 6	—	—	—
35 7	0/10[6]	19 6	—	—	—

[1] Number surviving at 13 weeks/number of animals per dose group For groups with no survivors, no final mean body weights or body weight changes are given
[2] Body weights were measured at Day 1 Subsequent calculations are based on animals surviving to the end of the study
[3] Mean weight change of the survivors
[4] (Dosed group mean/DMP control group mean) × 100
[5] Week of death 6 (10 animals killed due to severity of skin lesions)
[6] Week of death 1
[7] Week of death 1 (1 early death), 6 (9 animals killed due to severity of skin lesions)

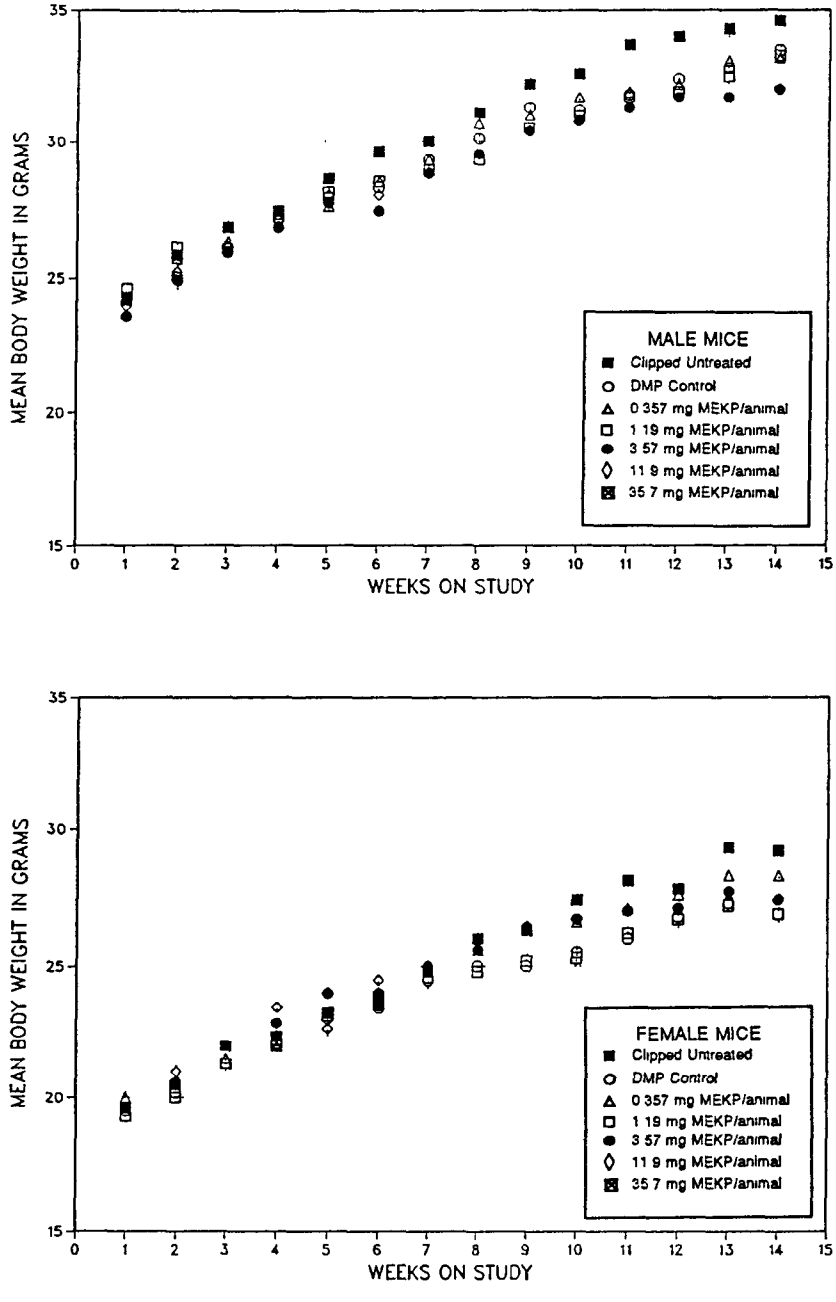

FIGURE 2 Body Weights of B6C3F$_1$ Mice Exposed to Methyl Ethyl Ketone Peroxide by Topical Administration for 13 Weeks

Changes in spleen weights of males and females treated with 3.57 mg MEKP/animal were considered related to treatment (Appendix A). Both absolute and relative spleen weights were significantly increased in these groups when compared to the DMP controls. However, the mean spleen weights of both male and female untreated control groups were greater than those of the DMP vehicle controls, which complicated interpretation of the MEKP effect (Table 9).

TABLE 9 Spleen and Body Weights of B6C3F$_1$ Mice Administered Methyl Ethyl Ketone Peroxide Topically for 13 Weeks[1]

			MEKP (mg/animal)		
	Untreated	DMP Control	0.357	1.19	3.57
MALE					
Necropsy body weight	35 2	33 8	32 9	32 2	31 8*
Spleen weight	0 077	0 067	0 072	0 068	0 078**
Relative spleen weight	2 23	1 99	2 18	2 11	2 45**
FEMALE					
Necropsy body weight	29 7**	26 7	28 5	26 8	27 3
Spleen weight	0 099**	0 078	0 084	0 083	0 100**
Relative spleen weight	3 35	2 94	2 99	3 10	3 67**

[1] Organ weights and body weights are given in grams, relative organ weights (organ-weight-to-body weight ratios) are given as mg organ weight/g body weight, n=10 Weights not given in 11 9 and 35 7 mg/animal groups due to 100% mortality in these groups

* Significantly different (P≤0 05) from the DMP control group by Williams' or Dunnett's test

** Significantly different (P≤0 01) from the DMP control group by Williams' or Dunnett's test

At necropsy, gross lesions were present at the application site in most MEKP treatment groups. High-dose mice (35.7 mg/animal), which died during Week 1, exhibited thickened skin with accumulation of subcutaneous fluid (edema) at the application site. Necropsy of mice in the 11.9 mg/animal group that were killed early due to severe skin lesions at the application site confirmed the presence of thickened skin with adherent crusts. Scaliness at the application site was observed in most mice in the 1.19 and 3.57 mg/animal groups. Splenic enlargement in several females from the 11.9 mg/animal (early sacrifice) group was also considered to be treatment related.

Microscopic lesions attributable to MEKP treatment were found at the application site; in general, these lesions were characterized by severe necrotic and exudative changes at the

higher dose levels and by mild hyperplastic changes at lower dose levels. In the animals that died during the first week, skin at the application site exhibited coagulative necrosis involving both epidermis and dermis, with minimal to moderate serosuppurative exudation in the dermis and subcutis. At the 3.57 and 11.9 mg MEKP/animal levels, smaller areas of dermal/epidermal coagulative necrosis were intermingled with more focal ulcerations, in which necrosis was confined to the epidermis. Epidermal hyperplasia (acanthosis) was present at the margins of ulcerated/necrotic areas except in those mice that died during Week 1. Minimal to mild acanthosis was the only significant microscopic finding in the 0.357 and 1.19 mg MEKP/animal groups; this finding was also present in several of the DMP control mice. Inflammation in the animals that survived past the first week was characterized by superficial neutrophilic exudate, which commonly mixed with necrotic debris to form crusts covering ulcerated areas, as well as by deeper dermal fibrovascular proliferation ("granulation tissue") beneath denuded skin.

The spleen and femoral bone marrow also exhibited effects considered secondary to the skin inflammation and necrosis. In the spleen, increased hematopoietic cell proliferation was present in all mice that died or that were killed before the end of the study (11.9 and 35.7 mg/animal groups). An additional finding in the spleen was the presence of increased amounts of golden-brown, intracellular pigment, presumably hemosiderin, in the red pulp of most mice in the 3.57 and 11.9 mg/animal groups. Hematopoietic cell hyperplasia, primarily of the myeloid series, was a common finding in the bone marrow in the 3.57, 11.9, and 35.7 mg/animal groups and was present in mice that died early and in those that survived to the end of the study. The following lesions, which were attributed to stress and/or moribund condition, were found only in mice that died during the first week: lymphoid necrosis and depletion in the spleen and thymus; centrilobular coagulative necrosis of the liver; cytoplasmic alteration of myocardial cells; and seminiferous tubule degeneration with syncytial cell formation in the testis. Table 10 summarizes the incidence and severity of selected lesions in mice from the 13-week dermal study of MEKP.

TABLE 10 Incidence[1] and Severity[2] of Selected Treatment-Related Lesions in B6C3F$_1$ Mice Administered Methyl Ethyl Ketone Peroxide Topically for 13 Weeks

			MEKP (mg/animal)				
	Untreated	DMP Control	0.357	1.19	3.57	11.9	35.7
MALE							
Skin (SOA)[3]							
Coagulative necrosis	0	0	0	0	2 (1.0)	0	10 (3.1)
Ulceration	0	0	0	0	2 (1.0)	7 (2.6)	10 (1.7)
Acanthosis	0	2 (1.0)	7 (1.3)	10 (1.4)	10 (1.8)	10 (2.0)	0
Spleen							
Hematopoietic cell proliferation	0	0	0	0	0	10 (2.1)	10 (3.0)
Pigment	0	0	0	0	10 (1.3)	10 (2.9)	0
Bone Marrow							
Myeloid hyperplasia	0	0	0	0	10 (2.4)	10 (3.2)	10 (3.0)
FEMALE							
Skin (SOA)							
Coagulative necrosis	0	0	0	0	1 (1.0)	7 (2.6)	10 (2.3)
Ulceration	0	0	0	0	1 (1.0)	6 (2.8)	8 (1.1)
Acanthosis	0	4 (1.0)	10 (1.0)	10 (1.9)	10 (2.5)	8 (2.1)	0
Spleen							
Hematopoietic cell proliferation	0	0	0	0	0	10 (2.8)	10 (2.6)
Pigment	0	0	0	0	10 (2.5)	9 (2.9)	0
Bone Marrow							
Myeloid hyperplasia	0	0	0	0	7 (1.6)	10 (2.9)	7 (1.8)

[1] Incidence is the number of animals with lesions from groups of 10
[2] Average severity score ( ) was based on the number of animals with lesions, 1 = minimal, 2 = slight, 3 = mild, 4 = moderate, 5 = marked
[3] SOA = site of application

Sperm morphology and vaginal cytology evaluations were performed on mice in the DMP control, 0.357, 1.19, and 3.57 mg MEKP/animal groups. None of the parameters examined were affected by MEKP treatment when compared to the DMP control (Appendix B).

Genetic Toxicity Studies

Methyl ethyl ketone peroxide (1 to 333 µg/plate) in dimethyl sulfoxide was not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, or TA98, with or without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 when tested using a preincubation protocol (Mortelmans *et al.*, 1986; Appendix C, Table C1). However, positive responses were obtained in tests with rodent cells *in vitro*. MEKP (2 to 10 nL/mL) in ethanol induced trifluorothymidine-resistant cells in the mouse lymphoma L5178Y test

in the absence of S9 activation (Table C2). The chemical was not tested with S9 in this assay. In cytogenetic tests with Chinese hamster ovary cells, MEKP in dimethyl sulfoxide induced sister chromatid exchanges (SCEs) (Table C3) and chromosomal aberrations (Abs) (Table C4) at doses up to 50 µg/mL with and without Aroclor 1254-induced male Sprague-Dawley rat liver S9. In the SCE test, a clearly positive, dose-related response was obtained in the absence of S9; with S9, a positive response was seen only at the highest nonlethal dose tested (50 µg/mL). Similarly, in the Abs test with S9, a positive response was seen only at the highest dose tested, which also produced observable toxicity.

The vehicle, dimethyl phthalate (DMP), at a purity of 99%, was not mutagenic in *S. typhimurium* when tested at doses up to 333 µg/plate under the same conditions as MEKP (Zeiger *et al.*, 1985). There was no induction of chromosomal aberrations in CHO cells at doses up to 1500 µg/mL without S9 and up to 5100 µg/mL with S9 (Loveday *et al.*, 1990). SCEs were induced at doses of 1240 to 2960 µg/mL in the presence of S9; DMP did not induce SCEs without S9 when tested at doses up to 1510 µg/mL (Loveday *et al.*, 1990).

Despite the positive chromosomal aberrations test results with MEKP in rodent cells *in vitro*, no increase was observed in the frequency of micronucleated erythrocytes in peripheral blood samples obtained from male and female mice at the end of the 13-week dermal toxicity study (Table C5). In addition, no alterations of the PCE/NCE ratio were seen, which implies that no overt toxicity was produced in the bone marrow cells by topical application of 0.357 to 3.57 mg MEKP/animal.

Although the pattern of genetic toxicity responses with MEKP is similar to that seen with DMP, the positive SCE results obtained with MEKP in DMP do not appear to be a result of the high concentration of DMP. SCEs were induced by MEKP in DMP at doses of 10 and 15 µg/mL (equivalent to 5.5 and 8.25 µg/mL DMP) (Appendix C; Table C3), whereas no effect was seen at doses below 1240 µg/mL of DMP alone (Loveday *et al.*, 1990).

DISCUSSION

MEKP is a potent oxidizing agent that has been reported to induce localized necrotic effects in animals following topical or parenteral administration and in humans following accidental exposure. The toxic effects of MEKP in DMP administered topically to rats and mice were primarily limited to inflammation and necrosis at the application site.

In the 2-week studies, MEKP at doses of up to 810 mg/kg body weight in rats and 1800 mg/kg body weight in mice, applied daily, caused dose-related increases in epidermal and dermal necrosis and inflammation, with some evidence of epidermal regeneration in animals that survived to the end of the studies. Lesions considered secondary to the dermal injury included hematopoietic cell proliferation in the spleen in rats and mice and myeloid hyperplasia of the sternebral bone marrow in mice. Treated mice also showed a marked increase in liver weight, and decreased thymus weights were generally noted in treated rats and mice. The dermal lesions were sufficiently severe to result in the death of some mice, but the pattern of deaths was not clearly dose related. Likewise, the pattern of weight gains of mice did not show a strong dose response. These 2 factors, plus the evidence for epidermal regeneration despite continued dosing, led to the selection of doses for the 13-week studies that were, in retrospect, excessive.

The high doses used for the 13-week studies (107 mg MEKP/rat and 35.7 mg MEKP/mouse) were nearly the same as the high doses (given on a mg/kg body weight basis) in the 2-week studies. However, because the range of doses for the 13-week studies was wider and included doses that were lower than those administered in the 2-week studies, the highest dose groups that contained survivors in the 13-week studies (10.7 mg/rat and 3.57 mg/mouse) received doses similar to those administered to the low-dose groups in the shorter studies. The dermal lesions produced in the 2 studies were similar. Necrosis was severe in rats and mice in the 2 highest dose groups (35.7 and 107 mg/rat, 11.9 and 35.7 mg/mouse) in the 13-week studies, resulting in the death of all high-dose mice, 2 high-dose female rats, and 1 female mouse in the 11.9 mg/animal group. The severe necrosis necessitated the early termination of the surviving animals in these dose groups. A no-observed-adverse-effect level (NOAEL) was not reached, with

acanthosis at the application site occurring in mice given doses as low as 0.357 mg and acanthosis and hyperkeratosis occurring in rats given as little as 1.07 mg. The highest dose groups of rats and mice allowed to continue to the end of the studies (10.7 mg/rat and 3.57 mg/mouse) showed focal ulcerations, inflammation, and fibrovascular proliferation at the application site. Thus, these studies were considered adequate for the assessment of the potential for systemic toxicity under conditions of repeated dermal contact both on ulcerated and nonulcerated skin.

Evidence for systemic injury under these conditions was limited. As in the 2-week studies, hematopoietic cell proliferation was noted in the spleen, and myeloid hyperplasia occurred in the bone marrow, primarily in animals that were killed early in the studies; both were considered secondary responses to the dermal lesions. Organ weight changes in the 13-week studies were not marked, and the substantial and unexplained increases in liver weights noted in mice in the 2-week studies were not clearly evident following the prolonged but relatively low-dose exposure of the 13-week studies.

The results of these studies suggest that significant amounts of MEKP in DMP do not become systemically available and that, as expected, the primary toxicity associated with contact with these chemicals is limited to the application site. The results of these studies are in general agreement with study results obtained for other members of this class of peroxides and are supported by findings of the clinical studies of humans exposed to MEKP by ingestion or by contact with the eyes or skin.

MEKP is one of a limited number of peroxides and hydroperoxides that have been tested for mutagenicity by the NTP; MEKP was not mutagenic in *S. typhimurium* strains TA100, TA1535, TA1537, or TA98, with or without metabolic activation. One would expect that these chemicals would yield reactive oxygen moieties that would be mutagenic in *Salmonella*. Hydrogen peroxide is a weak mutagen in *Salmonella* (Abu-Shakra and Zeiger, 1990), and mixed results have been reported for other peroxides and hydroperoxides (Haworth *et al.*, 1983; Mortelmans *et al.*, 1986; Zeiger, *et al.*, 1988). Additionally, where such peroxides have been reported as mutagenic in *Salmonella*, the response has usually been weak. Approximately half of the compounds in this class of chemicals that have been tested by the NTP have been mutagenic in *Salmonella*.

It is known from early studies performed by Ames and his coworkers (McCann *et al.*, 1975) and from the more recent studies carried out by the NTP (Ashby and Tennant, 1988, 1991; Zeiger *et al.*, 1990) that a positive response in the *Salmonella* mutagenicity test has a high (approximately 89%) positive predictivity for rodent carcinogenicity. However, nonmutagenicity in *Salmonella* is not predictive of noncarcinogenicity in rodents, and the *Salmonella* test is not responsive to approximately half of the chemicals found to be carcinogenic in rodents. The predictivity of the *S. typhimurium* test also varies with chemical class (Ashby and Tennant, 1991; Zeiger, 1987). Unfortunately, too few peroxides and hydroperoxides have been adequately tested in both the *S. typhimurium* and rodent carcinogenesis assays to draw any conclusions of the predictive value of *S. typhimurium* results for this class of chemicals. The predictive value of rodent bone marrow cytogenetics tests for carcinogenesis is under investigation, so it is not yet possible to draw any conclusions from the negative micronucleus test.

MEKP showed weak tumor-promoting activity when applied topically to the skin of mice that had been UV-irradiated (Logani *et al.*, 1984). The MEKP dose used in that study, 10 µg/mouse, was much lower than the low dose of 357 µg/mouse used in the present study. In general, the human exposure occurring in the workplace is through splashing, thereby affecting the skin. Presumably, because of the irritating properties of MEKP, it is immediately removed by washing. Therefore, it is not likely that sustained exposure of the type needed to promote skin tumor risk factors, as occurs with sunlight or with other chemicals, would occur with MEKP.

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APPENDIX A

Organ Weights and Organ-Weight-to-Body-Weight Ratios

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Dermal Study of Methyl Ethyl Ketone Peroxide[1]

	Untreated Control	DMP[2] Control	MEKP (mg/animal) 1.07	3.57	10.7
MALE					
n	10	10	10	10	10
Necropsy body weight	370 ± 5**	346 ± 6	365 ± 7	355 ± 7	302 ± 6**
Brain					
Absolute	1 982 ± 0 015	1 946 ± 0 012	1 984 ± 0 021	1 972 ± 0 014	1 938 ± 0 026
Relative	5 36 ± 0 06*	5 64 ± 0 10	5 44 ± 0 08	5 59 ± 0 14	6 43 ± 0 15**
Heart					
Absolute	1 054 ± 0 021	1 008 ± 0 019	1 088 ± 0 029	1 074 ± 0 016	0 960 ± 0 026
Relative	2 85 ± 0 05	2 92 ± 0 04	2 98 ± 0 05	3 03 ± 0 03	3 18 ± 0 08**
Right kidney					
Absolute	1 303 ± 0 031	1 268 ± 0 031	1 401 ± 0 041	1 328 ± 0 028	1 193 ± 0 022
Relative	3 52 ± 0 06	3 67 ± 0 08	3 83 ± 0 07	3 76 ± 0 10	3 95 ± 0 08*
Liver					
Absolute	14 553 ± 0 327	13 717 ± 0 423	14 732 ± 0 510	13 632 ± 0 333	11 050 ± 0 223**
Relative	39 34 ± 0 65	39 74 ± 1 37	40 28 ± 0 91	38 46 ± 0 58	36 56 ± 0 41*
Lungs					
Absolute	1 708 ± 0 075**	1 415 ± 0 033	1 638 ± 0 094	1 566 ± 0 053	1 383 ± 0 064
Relative	4 62 ± 0 21*	4 09 ± 0 09	4 48 ± 0 24	4 42 ± 0 12	4 58 ± 0 22
Spleen					
Absolute	0 786 ± 0 018**	0 711 ± 0 017	0 765 ± 0 023	0 722 ± 0 010	0 637 ± 0 019**
Relative	2 12 ± 0 04	2 06 ± 0 05	2 09 ± 0 04	2 04 ± 0 02	2 11 ± 0 07
Right testis					
Absolute	1 594 ± 0 029**	1 479 ± 0 025	1 539 ± 0 032	1 555 ± 0 025	1 476 ± 0 017
Relative	4 31 ± 0 05	4 29 ± 0 10	4 22 ± 0 05	4 39 ± 0 05	4 89 ± 0 07**
Thymus					
Absolute	0 359 ± 0 014	0 339 ± 0 011	0 378 ± 0 018	0 393 ± 0 014	0 266 ± 0 012**
Relative	0 97 ± 0 03	0 98 ± 0 03	1 03 ± 0 04	1 11 ± 0 04	0 88 ± 0 05
FEMALE					
n	10	10	10	10	10
Necropsy body weight	217 ± 4	209 ± 4	203 ± 4	205 ± 6	198 ± 3
Brain					
Absolute	1 786 ± 0 017	1 815 ± 0 011	1 837 ± 0 021	1 827 ± 0 024	1 825 ± 0 032
Relative	8 26 ± 0 15	8 70 ± 0 17	9 10 ± 0 20	8 95 ± 0 22	9 23 ± 0 16
Heart					
Absolute	0 778 ± 0 066	0 771 ± 0 016	0 775 ± 0 030	0 777 ± 0 016	0 728 ± 0 019
Relative	3 59 ± 0 29	3 69 ± 0 07	3 82 ± 0 12	3 80 ± 0 09	3 68 ± 0 09
Right kidney					
Absolute	0 805 ± 0 018	0 843 ± 0 022	0 842 ± 0 019	0 866 ± 0 021	0 858 ± 0 028
Relative	3 72 ± 0 08**	4 03 ± 0 07	4 17 ± 0 10	4 23 ± 0 10	4 33 ± 0 10*
Liver					
Absolute	7 699 ± 0 169	8 188 ± 0 314	7 314 ± 0 158**	7 408 ± 0 177*	7 124 ± 0 161**
Relative	35 52 ± 0 43*	39 14 ± 1 41	36 15 ± 0 59*	36 15 ± 0 61*	35 97 ± 0 55*

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Dermal Study of Methyl Ethyl Ketone Peroxide (continued)

	Untreated Control	DMP Control	MEKP (mg/animal) 1.07	3.57	10.7
FEMALE (continued)					
Lungs					
Absolute	1 157 ± 0 037	1 104 ± 0 045	1 125 ± 0 029	1 131 ± 0 040	1 069 ± 0 020
Relative	5 34 ± 0 15	5 27 ± 0 16	5 56 ± 0 12	5 52 ± 0 18	5 40 ± 0 09
Spleen					
Absolute	0 536 ± 0 015*	0 492 ± 0 013	0 479 ± 0 014	0 481 ± 0 010	0 481 ± 0 015
Relative	2 47 ± 0 06	2 35 ± 0 06	2 37 ± 0 06	2 35 ± 0 04	2 43 ± 0 05
Thymus					
Absolute	0 292 ± 0 011	0 285 ± 0 014	0 286 ± 0 011	0 273 ± 0 012	0 270 ± 0 015
Relative	1 35 ± 0 05	1 36 ± 0 06	1 41 ± 0 05	1 32 ± 0 03	1 36 ± 0 06

[1] Organ weights and body weights are given in grams, relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error) Weights not given in 35 7 and 107 mg/animal groups due to 100% mortality in these groups

[2] DMP = dimethyl phthalate

* Significantly different (P≤0 05) from the DMP control group by Williams' or Dunnett's test

** Significantly different (P≤0 01) from the DMP control group by Williams' or Dunnett's test

TABLE A2 Organ Weights and Organ-Weight-to-Body Weight Ratios for B6C3F$_1$ Mice in the 13-Week Dermal Study of Methyl Ethyl Ketone Peroxide[1]

	Untreated Control	DMP[2] Control	MEKP (mg/animal) 0.357	1.19	3.57
MALE					
n	10	10	10	10	10
Necropsy body weight	35 2 ± 0 9	33 8 ± 0 8	32 9 ± 0 6	32 2 ± 0 3	31 8 ± 0 5*
Brain					
Absolute	0 443 ± 0 004	0 447 ± 0 005	0 453 ± 0 006	0 443 ± 0 005	0 452 ± 0 004
Relative	12 68 ± 0 39	13 31 ± 0 36	13 79 ± 0 28	13 75 ± 0 12	14 23 ± 0 15*
Heart					
Absolute	0 154 ± 0 003	0 158 ± 0 004	0 152 ± 0 002	0 156 ± 0 005	0 149 ± 0 002
Relative	4 39 ± 0 09	4 70 ± 0 18	4 62 ± 0 08	4 84 ± 0 14	4 69 ± 0 07
Right kidney					
Absolute	0 322 ± 0 009	0 304 ± 0 009	0 311 ± 0 010	0 310 ± 0 009	0 304 ± 0 007
Relative	9 21 ± 0 37	9 02 ± 0 21	9 45 ± 0 27	9 62 ± 0 26	9 56 ± 0 19
Liver					
Absolute	1 687 ± 0 045	1 617 ± 0 039	1 648 ± 0 039	1 495 ± 0 013*	1 613 ± 0 035
Relative	47 96 ± 0 45	47 94 ± 0 81	50 09 ± 1 07	46 41 ± 0 36	50 72 ± 0 94
Lungs					
Absolute	0 182 ± 0 006	0 180 ± 0 008	0 199 ± 0 013	0 186 ± 0 009	0 175 ± 0 010
Relative	5 21 ± 0 23	5 39 ± 0 35	6 04 ± 0 36	5 78 ± 0 30	5 51 ± 0 34
Spleen					
Absolute	0 077 ± 0 005	0 067 ± 0 002	0 072 ± 0 004	0 068 ± 0 002	0 078 ± 0 002**
Relative	2 23 ± 0 20	1 99 ± 0 07	2 18 ± 0 09	2 11 ± 0 05	2 45 ± 0 06**
Right testis					
Absolute	0 119 ± 0 002	0 116 ± 0 003	0 119 ± 0 003	0 115 ± 0 003	0 115 ± 0 002
Relative	3 40 ± 0 10	3 44 ± 0 11	3 61 ± 0 07	3 56 ± 0 09	3 63 ± 0 04
Thymus					
Absolute	0 042 ± 0 003	0 041 ± 0 002	0 041 ± 0 003	0 037 ± 0 002	0 043 ± 0 004
Relative	1 20 ± 0 09	1 22 ± 0 07	1 25 ± 0 08	1 16 ± 0 06	1 37 ± 0 15
FEMALE					
n	10	10	10	10	10
Necropsy body weight	29 7 ± 0 5**	26 7 ± 0 5	28 5 ± 1 4	26 8 ± 0 5	27 3 ± 0 3
Brain					
Absolute	0 468 ± 0 004	0 463 ± 0 004	0 456 ± 0 005	0 463 ± 0 004	0 466 ± 0 004
Relative	15 81 ± 0 28**	17 42 ± 0 32	16 29 ± 0 71	17 31 ± 0 31	17 11 ± 0 17
Heart					
Absolute	0 137 ± 0 004	0 137 ± 0 005	0 134 ± 0 003	0 133 ± 0 003	0 145 ± 0 005
Relative	4 61 ± 0 09*	5 14 ± 0 19	4 75 ± 0 14	4 96 ± 0 08	5 32 ± 0 16
Right kidney					
Absolute	0 214 ± 0 005	0 222 ± 0 004	0 237 ± 0 005	0 235 ± 0 007	0 242 ± 0 009*
Relative	7 23 ± 0 20**	8 37 ± 0 29	8 43 ± 0 30	8 77 ± 0 23	8 88 ± 0 32
Liver					
Absolute	1 332 ± 0 023*	1 442 ± 0 034	1 298 ± 0 044*	1 308 ± 0 028	1 533 ± 0 046
Relative	44 97 ± 0 87**	54 33 ± 1 95	46 02 ± 1 70**	48 81 ± 0 78*	56 24 ± 1 55

TABLE A2 Organ Weights and Organ-Weight-to-Body Weight Ratios for B6C3F$_1$ Mice in the 13-Week Dermal Study of Methyl Ethyl Ketone Peroxide (continued)

	Untreated Control	DMP Control	MEKP (mg/animal) 0.357	1.19	3.57
FEMALE (continued)					
Lungs					
Absolute	0 195 ± 0 010	0 187 ± 0 009	0 230 ± 0 017*	0 192 ± 0 011	0 196 ± 0 008
Relative	6 57 ± 0 35	7 01 ± 0 27	8 12 ± 0 55	7 15 ± 0 36	7 20 ± 0 31
Spleen					
Absolute	0 099 ± 0 006**	0 078 ± 0 002	0 084 ± 0 003	0 083 ± 0 003	0 100 ± 0 003**
Relative	3 35 ± 0 22	2 94 ± 0 09	2 99 ± 0 13	3 10 ± 0 08	3 67 ± 0 09**
Thymus					
Absolute	0 054 ± 0 004	0 050 ± 0 004	0 055 ± 0 003	0 049 ± 0 002	0 051 ± 0 003
Relative	1 80 ± 0 13	1 88 ± 0 13	1 96 ± 0 13	1 83 ± 0 08	1 88 ± 0 09

[1] Organ weights and body weights are given in grams, relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error) Weights not given in 11 9 and 35 7 mg/animal dose groups due to 100% mortality in these groups

[2] DMP = dimetyhl phthalate

* Significantly different (P≤0 05) from the DMP control group by Williams' or Dunnett's test

** Significantly different (P≤0 01) from the DMP control group by Williams' or Dunnett's test

APPENDIX B

Reproductive Tissue Evaluations and Estrous Cycle Characterization

TABLE B1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Dermal Study of Methyl Ethyl Ketone Peroxide

Study Parameters[1]	DMP[2] Control	MEKP (mg/animal) 1.07	3.57	10.7
Weights (g)				
Necropsy body weight	346 ± 6	365 ± 7	355 ± 7	302 ± 6**
Right epididymis	0 451 ± 0 009	0 466 ± 0 013	0 466 ± 0 008	0 454 ± 0 006
Right cauda epididymis	0 162 ± 0 004	0 163 ± 0 006	0 160 ± 0 004	0 161 ± 0 007
Right testis	1 479 ± 0 025	1 539 ± 0 032	1 555 ± 0 025	1 476 ± 0 017
Spermatozoal measurements				
Motility (%)	75 ± 4	79 ± 3	74 ± 3	84 ± 2[3]
Concentration (10^6/g cauda epididymal tissue)	553 ± 42	652 ± 37	506 ± 27	586 ± 30
Abnormal sperm (%)	0 74 ± 0 09	0 87 ± 0 08	0 62 ± 0 11	0 60 ± 0 08

[1] Data presented as mean ± standard error, n=10, except where noted Differences from the control group for reproductive tissue weights are not significant by Dunnett's test, spermatozoal measurements are not significant by Dunn's or Shirley's test
[2] DMP = dimethyl phthalate
[3] n=9
** Significantly different (P≤0 01) from the control group by William's test

TABLE B2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Dermal Study of Methyl Ethyl Ketone Peroxide

Study Parameters[1]	DMP[2] Control	MEKP (mg/animal) 1.07	3.57	10.7
Necropsy body weight (g)	209 ± 4	203 ± 4	205 ± 6	198 ± 3
Estrous cycle length (days)	5 00 ± 0 00	5 00 ± 0 17[3]	5 00 ± 0 00	5 10 ± 0 10
Estrous stages as % of cycle				
Diestrus	32 9	38 6	28 6	41 4
Proestrus	20 0	8 6	20 0	17 1
Estrus	27 1	35 7	32 9	21 4
Metestrus	20 0	17 1	18 6	20 0

[1] Necropsy body weight and estrous cycle length data are presented as mean ± standard error, n=10, except where noted Differences from the control group for necropsy body weights are not significant by Williams' test, estrous cycle lengths are not significant by Dunn's or Shirley's test By multivariate analysis of variance (MANOVA), dosed groups do not differ significantly from controls in the relative length of time spent in the estrous stages
[2] DMP = dimethyl phthalate
[3] For 1/10 animals in the 1 07 mg/animal dose group, estrous cycle length was longer than 7 days or was unclear, data for these animals are not included in the mean

TABLE B3 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Dermal Study of Methyl Ethyl Ketone Peroxide

Study Parameters[1]	DMP[2] Control	MEKP (mg/animal) 0.357	1.19	3.57
Weights (g)				
Necropsy body weight	33 8 ± 0 8	32 9 ± 0 6	32 2 ± 0 3	31 8 ± 0 5*
Right epididymis	0 047 ± 0 002	0 046 ± 0 004	0 044 ± 0 001	0 044 ± 0 002
Right cauda epididymis	0 016 ± 0 001	0 014 ± 0 001	0 015 ± 0 001	0 016 ± 0 001
Right testis	0 116 ± 0 003	0 119 ± 0 003	0 115 ± 0 003	0 115 ± 0 002
Spermatozoal measurements				
Motility (%)	86 ± 2	84 ± 2	87 ± 1	83 ± 2
Concentration (10^6/g cauda epididymal tissue)	1233 ± 143	1308 ± 106	1387 ± 83	1404 ± 128
Abnormal sperm (%)	1 46 ± 0 17	1 24 ± 0 12	1 92 ± 0 26	1 26 ± 0 12

[1] Data presented as mean ± standard error, n=10 Reproductive tissue weights are not significant by Dunnett's test, spermatozoal measurements are not significant by Dunn's test
[2] DMP = dimethyl phthalate
* Significantly different (P≤0 05) from the control group by William's test

TABLE B4 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Dermal Study of Methyl Ethyl Ketone Peroxide

Study Parameters[1]	DMP[2] Control	MEKP (mg/animal) 0.357	1.19	3.57
Necropsy body weight (g)	26 7 ± 0 5	28 5 ± 1 4	26 8 ± 0 5	27 3 ± 0 3
Estrous cycle length (days)	4 60 ± 0 16	4 67 ± 0 17[3]	4 33 ± 0 24[3]	4 30 ± 0 15
Estrous stages as % of cycle				
Diestrus	25 7	18 6	27 1	22 9
Proestrus	28 6	25 7	28 6	24 3
Estrus	21 4	35 7	28 6	35 7
Metestrus	24 3	20 0	15 7	17 1

[1] Necropsy body weight and estrous cycle length data are presented as mean ± standard error, n=10, except where noted Differences from the control group for necropsy body weights are not significant by Dunnett's test, estrous cycle lengths are not significant by Dunn's or Shirley's test By multivariate analysis of variance (MANOVA), dosed groups do not differ significantly from controls in the relative length of time spent in the estrous stages
[2] DMP = dimethyl phthalate
[3] For 1/10 animals in the 0 357 and 1 19 mg/animal dose groups, estrous cycle length was longer than 7 days or was unclear, data for these animals are not included in the mean

APPENDIX C

Genetic Toxicology

TABLE C1 Mutagenicity of Methyl Ethyl Ketone Peroxide in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	170 ± 2 6	111 ± 0 7	143 ± 4 8	122 ± 6 4	137 ± 6 0	118 ± 8 6
	1	135 ± 9 8	121 ± 2 3				
	3	135 ± 5 8	115 ± 9 6	150 ± 2 1	123 ± 8 1	157 ± 3 2	134 ± 4 4
	10	136 ± 12 2	136 ± 7 1	138 ± 2 3	126 ± 7 8	139 ± 6 7	128 ± 1 5
	33	157 ± 10 3	130 ± 4 1	136 ± 4 7	123 ± 11 2	165 ± 7 9	131 ± 8 7
	100	146 ± 7 2	134 ± 8 7	136 ± 3 5	123 ± 4 7	153 ± 13 5	149 ± 1 5
	333			142 ± 16 8	136 ± 10 4	97 ± 23 4	190 ± 4 6
Trial summary		Negative	Negative	Negative	Negative	Negative	Equivocal
Positive control[3]		365 ± 4 7	313 ± 15 6	1,603 ± 66 1	1,015 ± 20 1	682 ± 17 3	675 ± 35 5
TA1535	0	26 ± 6 4	27 ± 1 2	10 ± 2 3	11 ± 3 3	18 ± 3 2	12 ± 2 0
	1	23 ± 2 2	27 ± 0 9				
	3	26 ± 5 3	24 ± 0 0	12 ± 1 9	8 ± 2 6	17 ± 1 9	14 ± 1 2
	10	24 ± 2 6	29 ± 0 9	9 ± 2 6	8 ± 2 5	15 ± 2 1	12 ± 1 7
	33	18 ± 3 5	25 ± 6 1	12 ± 2 4	7 ± 0 6	16 ± 2 0	12 ± 2 3
	100	16 ± 0 9	14 ± 1 0	10 ± 1 7	7 ± 2 3	18 ± 1 3	11 ± 1 2
	333			12 ± 1 9	8 ± 2 6	8 ± 3 0	10 ± 1 2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		359 ± 12 5	319 ± 12 3	369 ± 17 8	336 ± 28 7	198 ± 8 7	151 ± 1 8
TA1537	0	5 ± 1 2	6 ± 0 6	4 ± 0 9	6 ± 0 3	14 ± 1 9	11 ± 2 3
	1	7 ± 1 5	5 ± 0 3				
	3	5 ± 0 3	7 ± 1 5	12 ± 2 0	11 ± 2 4	13 ± 2 0	12 ± 1 5
	10	7 ± 1 0	5 ± 0 3	11 ± 1 5	6 ± 1 8	11 ± 1 8	14 ± 1 5
	33	7 ± 0 0	8 ± 1 2	11 ± 2 2	5 ± 0 9	14 ± 1 5	10 ± 1 2
	100	6 ± 1 7	4 ± 0 3[4]	6 ± 0 9	5 ± 0 9	11 ± 3 9	10 ± 1 7
	333			9 ± 1 9	6 ± 0 3[4]	11 ± 4 4	11 ± 2 5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		147 ± 18 7	74 ± 8 4	589 ± 2 0	313 ± 20 5	161 ± 15 3	159 ± 19 0
TA98	0	19 ± 1 5	18 ± 3 5	31 ± 3 5	29 ± 4 7	49 ± 4 1	29 ± 1 5
	1	20 ± 2 0	19 ± 1 0				
	3	21 ± 3 6	23 ± 4 6	28 ± 4 0	25 ± 7 7	41 ± 5 9	32 ± 2 3
	10	17 ± 0 9	24 ± 2 6	31 ± 3 2	27 ± 1 5	37 ± 2 3	25 ± 2 0
	33	13 ± 2 3	23 ± 1 3	36 ± 5 7	32 ± 1 9	32 ± 3 7	27 ± 3 6
	100	27 ± 1 2	1 ± 0 7[4]	30 ± 2 8	33 ± 2 7	42 ± 3 8	29 ± 3 4
	333			34 ± 3 5	32 ± 1 3	31 ± 5 3	24 ± 4 7
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		591 ± 76 8	520 ± 30 7	1,171 ± 136 5	764 ± 34 1	502 ± 49 5	522 ± 44 2

TABLE C1 Mutagenicity of Methyl Ethyl Ketone Peroxide in *Salmonella typhimurium* (continued)

[1] Study performed at SRI International The detailed protocol and these data are presented in Mortelmans *et al* (1986) Cells and methyl ethyl ketone peroxide or solvent (dimethylsulfoxide) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver High dose was limited by toxicity 0 µg/plate dose is the solvent control

[2] Revertants are presented as mean ± the standard error from 3 plates

[3] The positive controls in the absence of metabolic activation were 4-nitro-*o*-phenylenediamine (TA98), sodium azide (TA100 and TA1535), and 9 aminoacridine (TA1537) The positive control for metabolic activation with all strains was 2-aminoanthracene

[4] Slight toxicity

TABLE C2 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by Methyl Ethyl Ketone Peroxide[1]

Compound	Concentration	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[2]	Average Mutant Fraction
-S9						
Trial 1						
Ethanol						
		104	128	100	32	
		50	57	44	29	
		65	103	69	35	
		78	113	71	31	32
Methyl methanesulfonate (μg/mL)						
	5	57	41	505	295	
		52	59	448	284	
		63	66	417	219	266*
Methyl ethyl ketone peroxide (nL/mL)						
	2	72	60	136	63	
		40	44	123	103	
		39	38	111	94	87*
	3	57	51	88	52	
		29	25	88	99	
		35	40	88	83	78*
	4	30	39	143	158	
		46	32	273	200	
		61	60	385	211	190*
	6	39	55	229	198	
		66	38	390	197	
		70	75	319	152	182*
	8	61	34	408	222	
		59	19	396	223	223*
	10	84	29	481	191	
		65	23	495	254	222*

TABLE C2 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by Methyl Ethyl Ketone Peroxide (continued)

Compound	Concentration	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Trial 2						
Ethanol						
		118	114	118	33	
		95	98	127	45	
		85	88	132	52	43
Methyl methanesulfonate (µg/mL)						
	5	76	62	640	279	
		81	63	604	250	
		84	69	601	238	256*
Methyl ethyl ketone peroxide (nL\mL)						
	0 625	113	98	106	31	
		85	81	81	32	
		84	88	84	33	32
	1 25	91	99	108	39	
		107	92	106	33	
		91	100	98	36	36
	2 5	85	65	182	71	
		91	58	266	97	
		97	86	108	37	69*
	5	75	36	227	101	
		73	35	481	221	
		75	41	424	188	170*
	7 5	93	27	761	272	
		86	23	776	300	
		79	31	544	230	267*
	10	74	12	696	313	
		75	16	740	327	
		74	23	616	279	306*

[1] Study performed at Litton Bionetics, Inc The experimental protocol is presented in detail by Myhr *et al* (1985) The highest dose of methyl ethyl ketone peroxide was determined by toxicity All doses were tested in triplicate, the average of the three tests is presented in the table

[2] Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at MF/1 x 10^6 cells treated)

* Positive response ($P \leq 0.05$)

TABLE C3 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Methyl Ethyl Ketone Peroxide[1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%)[2]
-S9								
Trial 1								
Summary Weak Positive								
Dimethylsulfoxide		50	1,046	460	0 43	9 2	26 0	
Mitomycin-C								
	0 001	50	1,051	1,317	1 25	26 3	26 0	184 94
	0 01	10	209	619	2 96	61 9	26 0	573 47
Methyl ethyl ketone peroxide								
	0 5	50	1,050	489	0 46	9 8	26 0	5 90
	1 6	50	1,042	529	0 50	10 6	26 0	15 44
	5	50	1,050	519	0 49	10 4	26 0	12 40
	16	50	1,048	644	0 61	12 9	26 0	39 73*
	50	0[3]						
								P<0 001[4]
Trial 2								
Summary Positive								
Dimethylsulfoxide		50	1,047	450	0 42	9 0	26 5	
Mitomycin-C								
	0 005	50	1,049	2,115	2 01	42 3	26 5	369 11
	0 01	10	210	648	3 08	64 8	26 5	617 96
Methyl ethyl ketone peroxide								
	2	50	1,049	447	0 42	8 9	26 5	-0 85
	5	50	1,048	497	0 47	9 9	26 5	10 34
	10	50	1,048	591	0 56	11 8	26 5	31 21*
	15	50	1,051	657	0 62	13 1	26 5	45 45*
	20	0[3]						
								P<0 001

TABLE C3 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Methyl Ethyl Ketone Peroxide (continued)

Compound	Dose (µg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%)
+S9								
Trial 1								
Summary Weak Positive								
Dimethylsulfoxide		50	1,042	432	0 41	8 6	26 0	
Cyclophosphamide	0 3	50	1,040	701	0 67	14 0	26 0	62 58
	2	10	209	339	1 62	33 9	26 0	291 24
Methyl ethyl ketone peroxide	1 6	50	1,046	463	0 44	9 3	26 0	6 77
	5	50	1,042	461	0 44	9 2	26 0	6 71
	16	50	1,046	460	0 43	9 2	26 0	6 08
	50	50	1,039	615	0 59	12 3	26 0	42 77*
	160	0[3]						
								P<0 001
Trial 2								
Summary Weak positive								
Dimethylsulfoxide		50	1,042	461	0 44	9 2	26 0	
Cyclophosphamide	0 3	50	1,049	712	0 67	14 2	26 0	53 42
	2	10	209	394	1 88	39 4	26 0	326 11
Methyl ethyl ketone peroxide	10	50	1,038	441	0 42	8 8	26 0	-3 97
	25	50	1,042	487	0 46	9 7	26 0	5 64
	50	50	1,044	581	0 55	11 6	26 0	25 79*
	100	0[3]					31 0[5]	
								P<0 001

[1] Study performed at Environmental Health Research and Testing, Inc SCE=sister chromatid exchange, BrdU=bromodeoxyuridine A detailed description of the SCE protocol is presented by Galloway *et al* (1987)

[2] Percentage increase in SCEs/chromosome of culture exposed to methyl ethyl ketone peroxide relative to those of culture exposed to solvent

[3] Toxic, no scorable cells present

[4] Significance was tested by the linear regression trend test vs log of the dose

[5] Because methyl ethyl ketone peroxide induced a delay in the cell division cycle at this dose, harvest time was extended to maximize the proportion of second division cells available for analysis No viable cells were recovered

* Positive (>20% increase over solvent control)

TABLE C4 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Methyl Ethyl Ketone Peroxide[1]

-S9					+S9				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)
Trial 1 – Harvest time 12 0 hours Summary Weak positive					**Trial 1** – Harvest time 12 0 hours Summary Questionable				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	0	0 00	0 0		100	9	0 09	0 0
Mitomycin C					Cyclophosphamide				
0 25	100	53	0 53	40 0	15	100	55	0 55	40 0
1	100	72	0 72	48 0	50	50	72	1 44	68 0
Methyl ethyl ketone peroxide					Methyl ethyl ketone peroxide				
1 6	100	0	0 00	0 0	1 6	100	2	0 02	2 0
5	100	2	0 02	2 0	5	100	4	0 04	2 0
16	100	2	0 02	2 0	16	100	0	0 00	0 0
50	69[2]	10	0 14	7 0*	50	100	7	0 07	5 0*
					160	0[3]			
				P<0 001[4]					P=0 029
Trial 2 – Harvest time 12 0 hours Summary Positive					**Trial 2** – Harvest time 10 8 hours Summary Weak Positive				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	0	0 00	0 0		100	0	0 00	0 0
Mitomycin C					Cyclophosphamide				
0 25	100	31	0 31	23 0	15	100	26	0 26	25 0
1	50	43	0 86	50 0	50	50	54	1 08	32 0
Methyl ethyl ketone peroxide					Methyl ethyl ketone peroxide				
20	100	4	0 04	4 0	16	100	2	0 02	2 0
30	100	15	0 15	11 0*	25	100	2	0 02	2 0
40	100	26	0 26	16 0*	50	100	2	0 02	2 0
50	100	10	0 10	8 0*	75	100	7	0 07	6 0*
				P<0 001					P=0 008

[1] Study performed at Environmental Health Research and Testing, Inc Abs=aberrations A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway *et al* (1987)
[2] At 50 µg/mL, only 69 cells could be scored due to toxicity
[3] Toxic, no scorable cells present
[4] Significance of percent cells with aberrations tested by the linear regression trend test vs log of the dose
* Positive (P≤0 05)

TABLE C5 Frequency of Micronuclei in Peripheral Blood Erythrocytes of B6C3F$_1$ Mice Treated with Methyl Ethyl Ketone Peroxide for 13 Weeks[1]

Dose (mg/animal)	Micronucleated Cells/1,000 Cells		
	PCEs	NCEs	PCEs (%)
MALE			
Untreated control	2 41 ± 0 37	2 03 ± 0 23	2 25 ± 0 16
DMP[2] control	2 59 ± 0 68	1 74 ± 0 17	2 18 ± 0 10
0 357	2 31 ± 0 41	1 66 ± 0 18	2 04 ± 0 11
1 19	3 72 ± 0 75	2 21 ± 0 23	2 23 ± 0 20
3 57	1 90 ± 0 38	1 91 ± 0 22	2 13 ± 0 15
Trend test[3]	P=0 800	P=0 445	
ANOVA[4]			P=0 849
FEMALE			
Untreated control	1 90 ± 0 38	1 22 ± 0 16	1 94 ± 0 17
DMP control	1 89 ± 0 31	1 21 ± 0 13	1 97 ± 0 14
0 357	2 37 ± 0 41	1 25 ± 0 12	2 01 ± 0 21
1 19	1 61 ± 0 35	1 30 ± 0 16	2 46 ± 0 29
3 57[5]	1 99 ± 0 49	1 28 ± 0 10	2 04 ± 0 19
Trend test	P=0 536	P=0 514	
ANOVA			P=0 564

[1] PCE=polychromatic erythrocytes NCE=normochromatic erythrocytes Numbers represent mean ± standard error for groups of 10 animals unless otherwise noted
[2] DMP = dimethyl phthalate
[3] Cochran Armitage linear regression of proportions for PCEs or linear contrasts from analysis of variance for NCEs
[4] Analysis of variance on ranks
[5] n=9

NTP TECHNICAL REPORTS ON TOXICITY STUDIES PRINTED AS OF DECEMBER 1992

Toxicity Report Number	Chemical	Route of Exposure	Publication Number
1	Hexachloro-1,3-butadiene	Dosed Feed	91-3120
2	*n*-Hexane	Inhalation	91-3121
3	Acetone	Drinking Water	91-3122
4	1,2-Dichloroethane	Drinking Water, Gavage	91-3123
5	Cobalt Sulfate Heptahydrate	Inhalation	91-3124
6	Pentachlorobenzene	Dosed Feed	91-3125
7	1,2,4,5-Tetrachlorobenzene	Dosed Feed	91-3126
8	D & C Yellow No. 11	Dosed Feed	91-3127
9	*o*-Cresol *m*-Cresol *p*-Cresol	Dosed Feed	92-3128
10	Ethylbenzene	Inhalation	92-3129
11	Antimony Potassium Tartrate	Drinking Water, I.P. Inject.	92-3130
12	Castor Oil	Dosed Feed	92-3131
13	Trinitrofluorenone	Dermal, Dosed Feed	92-3132
14	*p*-Chloro-α,α,α-Trifluorotoluene	Gavage (corn oil, a-CD)	92-3133
15	*t*-Butyl Perbenzoate	Gavage	92-3134
16	Glyphosate	Dosed Feed	92-3135
17	Black Newsprint Ink	Dermal	92-3340
19	Formic Acid	Inhalation	92-3342
20	Diethanolamine	Drinking Water, Dermal	92-3343
21	2-Hydroxy-4-Methoxybenzophenone	Dosed Feed, Drinking Water	92-3344
22	N, N-Dimethylformamide	Inhalation	93-3345
23	*o*-Nitrotoluene *m*-Nitrotoluene *p*-Nitrotoluene	Dosed Feed	92-3346