National Toxicology Program
Toxicity Report Series
Number 17

NTP Technical Report on Toxicity Studies of

Black Newsprint Inks

Administered Topically to F344/N Rats and C3H Mice

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Post Office Box 12233
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July 1992

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

NOTE TO THE READER

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The 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.

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

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CONTRIBUTORS

The NTP report on the toxicity studies of black newsprint ink is based primarily on 30-day and 13-week studies performed over the period of December, 1987, to September, 1988, at Battelle Memorial Laboratories, Columbus Division, Columbus, Ohio.

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ABSTRACT

Toxicity studies were conducted by applying black newsprint inks or mineral oils to clipped skin of the dorsal interscapular area of C3H mice and F344/N rats of both sexes, to determine systemic and local effects. Four lots of both letterpress and offset types of newsprint ink were studied, either as composite mixtures or as individual lots. An industrial grade mineral oil, used as an extender for newsprint ink formulation, and USP medicinal grade mineral oil also were studied. Analyses for the presence of polycyclic aromatic hydrocarbons (PAHs) were conducted on composite ink mixtures and mineral oils; letterpress and offset ink mixtures were found to have cumulative concentrations of 206 and 105 ppm, respectively; the concentration of PAHs in the printing ink mineral oil sample was 208 ppm, while none were detected in the USP grade mineral oil. In genetic toxicity studies, letterpress and offset newsprint ink composite mixtures were each mutagenic in *Salmonella typhimurium* strains TA98 and TA100 when tested in a preincubation protocol with added hamster liver S9. With rat liver S9, results for both inks were positive in strain TA98 and negative in strain TA100. Neither type of ink was mutagenic in the absence of S9 activation.

In 30-day studies, 5 rats and mice per sex were given single, daily dermal applications of letterpress or offset newsprint inks, 5 days per week, for a total of 21 - 22 applications. Dose groups for each type of ink received either the neat (undiluted) composite ink mixture, or the 3:1, 1:1, or 1:3 dilutions (ink:USP mineral oil), with a total dose volume of 100 (mice) or 250 (rats) µl. All animals survived until the end of the studies. Toxicity attributed to ink administration was limited to decreased body weight gains in female rats treated with neat and the 3:1 dilution of letterpress ink, and to scaliness at the site of application in 1 or more mice in each letterpress ink treatment group. As a result of grooming activity and the large amount of test chemical applied, chemicals were spread over the body, and there was evidence that some oral ingestion had occurred.

In 13-week studies, various ink and mineral oil formulations were administered dermally to 10 rats and mice per sex. To prevent accumulation of inks and distribution over the body as seen in the 30-day studies, the frequency of application was reduced to twice weekly and the total dose volume was decreased to 20 microliters for mice and 50 microliters for rats. Treatment groups for rats consisted of letterpress ink mixture, offset ink mixture, printing ink mineral oil, USP mineral oil, and clipped, untreated controls. Groups of mice were administered each of the 4 individual lots of both letterpress and offset inks, the composite mixtures of each, and printing ink and USP mineral oils; clipped, untreated groups served as controls. All rats, all male mice, and all female mice except one administered offset ink-lot E survived to the end of the studies. Effects attributable to compound administration in rats were limited to decreased body weight gains in females treated with printing ink mineral oil and letterpress ink mixture, and increased liver and kidney weights in both males and females exposed to USP mineral oil; there were no local toxic effects at the site of application. In mice, there were no body weight effects, but liver weights were increased in most ink and mineral oil treatment groups of both sexes. Dermal toxicity was evidenced in mice by scaliness and irritation at the site of application of both sexes treated with USP mineral oil and letterpress ink-lot C. Microscopically, local toxicity at the site of application was observed in mice of all treatment groups and was characterized by acanthosis and inflammation.

In summary, results of these studies indicate that topical administration of black newsprint inks and mineral oils produces local toxicity at the site of application in mice; toxic effects on the skin in this species are consistent with those of a primary cutaneous irritant. In rats, possible evidence for toxicity was limited to decreased body weight gains in females treated with letterpress ink formulations.

PEER REVIEW

Peer Review Panel

The members of the peer review panel who evaluated the draft report on the toxicity studies on black newsprint inks on July 10, 1991, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members act to determine if the design and conditions of the NTP studies were appropriate and to ensure that the toxicity study report fully and clearly presents the experimental results and conclusions.

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Summary of Peer Review Comments

On July 9 and 10, 1991, 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 black newsprint inks.

Dr. J.F. Mahler, NIEHS, introduced the short-term toxicity studies of black newsprint inks by reviewing the uses of and rationale for study the inks, their composition, the methodology for topical application of the inks and mineral oils, experimental design, and results.

Dr. Davis, a principal reviewer, said the study was well-designed, very complex, and had been concisely and clearly reported. He said that the report summary mentioned little if any toxicity beyond the site of topical application, yet female rats had decreased weight gains and decreased heart and lung weights, compared to controls. He asked if these effects might be attributable to decreased feed consumption. Dr. Mahler agreed that the body-weight decreases were substantial enough to be seen as evidence of toxicity and that this would be noted in the report.

Dr. Bailey, a second principal reviewer, said the experiments were well-done and the report well-written. He cited references to the work of Blackburn *et al.* on the modified Ames assay and said these references should be included in the report, since the assay can accurately differentiate between carcinogenic and non-carcinogenic mineral oils and would also provide highly reliable estimates of the relative potency of such materials in mouse skin painting bioassays. Dr. Mahler said a later reference to the modified Ames assay was cited in the report, but that the Blackburn *et al.* work would be included as well.

Dr. Bailey said it would be helpful to include the refining histories for the printing ink mineral oils used in the study, since reference was made to the fact that less-refined oils have greater carcinogenic potential. Dr. Mahler said he would seek to include the refining histories of these oils in an appendix; however, subsequent efforts to locate further information concerning the refining histories were unsuccessful.

Dr. Bailey asked why the C3H mouse was used in the study. Dr. J. Bucher, NIEHS, noted that the National Institute of Occupational Safety and Health (NIOSH) had suggested use of that strain, saying it had a data base on dermal application studies using that type of animal. However, Dr. J. Haartz, NIOSH, said this database was not large and that much of it was based on studies involving asphalt fumes and fume fractions.

Mr. Beliczky said reference should be made to the fact that carbon black, used in some black newsprint inks, contains polycyclic aromatic hydrocarbons (PAHs); he noted that the International Agency for Research on Cancer (IARC) lists some PAHs in oil as human skin carcinogens. Dr. Mahler said that references cited in the report confirm Mr. Beliczky's comments.

After discussion of editorial matters, the panel agreed to accept the report, with the indicated changes.

INTRODUCTION

Physical Properties, Production, and Exposure

Black newsprint inks are viscous to semi-solid mixtures of carbon black, petroleum pitch, and assorted grades of petroleum-based oils and additives. There are 2 newspaper printing processes, letterpress and offset, accounting for 53% and 47% of newspapers printed, respectively. Inks used in each process have different compositions, as seen below in figures provided by the National Association of Printing Ink Manufacturers (NAPIM) (NIOSH, 1981):

	Offset	Letterpress
Carbon Black	16-18%	10-12%
Hydrocarbon varnish	12-15%	-
High boiling aliphatic oil	5-10%	-
Flow agent or black ink oil	2-5%	2-2.5%
Mineral oil	52-65%	85.5-88%

There are 4 U.S. manufacturers of black newsprint inks; each producer prepares a new batch for distribution approximately once a month. The actual composition of the inks may vary slightly from batch to batch and also from manufacturer to manufacturer; thus, there is no single representative batch of black newsprint ink. Summary data from NAPIM indicate that in 1981, 2.25×10^8 pounds (1.02×10^8 kg) of letterpress ink and 1×10^8 pounds (4.54×10^7 kg) of offset ink were produced.

Human exposure to black newsprint inks may occur by skin contact, inhalation of ink-oil mists, or ingestion. NIOSH estimates that 165,000 workers in 8200 major establishments currently are exposed to black newsprint inks (NIOSH, 1981). The major route of exposure in the workplace is dermal, and there is potential widespread dermal exposure to the public from "rub-off" of newsprint ink on the reader's fingers. Inhalation of ink mists also is an occupational hazard. Although there are no reported OSHA exposure limits for black newsprint inks, the standard for mineral oil mist is 5 mg/m^3, averaged over an 8-hour workshift. Exposure to ink mists in the workplace has decreased in recent years, due to innovations in printing methods. A 1980 survey of the New York Times pressroom found airborne ink mist levels were less than 1 mg/m^3, reduced by a factor of 10 from comparable data in a 1970 survey (Nicholson, 1980).

Epidemiology

Numerous epidemiologic studies have demonstrated a clear association of occupational exposure to polycyclic aromatic hydrocarbons (PAHs) and/or petroleum products, with an increased incidence of cancer (Bingham *et al.,* 1980; IARC, 1984). Mineral oil and carbon black, two of the major petrochemical components of black newsprint inks, have been examined individually in many of these studies. Exposure to mineral oils used in a variety of occupations has been associated consistently with the occurrence of squamous cell carcinomas of the skin. There are no conclusive studies to indicate a similar carcinogenic hazard from occupational exposure to carbon black. With particular regard to the printing industry, newspaper pressmen are exposed to printing inks dermally, as well as to ink-oil mists generated from lubricating oils and from printing inks which

contain mineral oil (a vehicle for carbon black). An early epidemiologic survey (Ask-Upmark,1955) indicated a marked increase in the incidence of lung cancer among Stockholm printing workers. Subsequent studies examining the printing industry have either supported the evidence for increased risk of lung cancer (Greenberg, 1972; Moss *et al.,* 1972; Nicholson *et al.,* 1981) or shown no significant differences from control populations (Pasternack and Ehrlich, 1972; Goldstein *et al.,* 1970). Evidence for increased cancer of the oral cavity (Nicholson *et al.,* 1981; Lloyd *et al.,* 1977), hematopoietic system (Lloyd *et al.,* 1977; Greene *et al.,* 1979; Paganini-Hill *et al.*, 1980; Zoloth *et al.,* 1986), and urinary bladder (Coggon *et al.,* 1984; Blot and Fraumeni, 1978) have been reported in printing pressmen. An increased incidence of malignant melanoma is also reported for workers in the printing industry (McLaughlin *et al.,* 1988). Contact dermatitis is recognized as an occupational hazard in both the petroleum industry (Birmingham, 1988) and in the printing industry (Nethercott, 1988).

Animal Studies

Extensive experimental data demonstrate the carcinogenic potential of PAHs and/or petroleum products (Bingham *et al.,* 1980; IARC, 1984), including many studies of mineral oil and carbon black, the major components of black newsprint inks. Generally, animal data show that the carcinogenicity of a particular mineral oil is related to its refining history; less refined oils have a greater carcinogenic potential (Bingham, 1988), presumably because they have larger amounts of PAHs. For example, Doak *et al.* (1983) applied 12 variously refined mineral oils in 0.25 ml volumes to the dorsal skin of CF-1 mice for up to 78 weeks; epithelial tumors of the treated skin developed after application of 6 of the 12 oils, with the least refined oils inducing the highest tumor response. Another noteworthy finding of this study was the development of irritative dermatitis, with significant epidermal hyperplasia, in 10 of the 12 oil treatment groups; however, the authors concluded that there was no correlation between the potential to induce hyperplasia and tumorigenic potential. Available data from animal studies with carbon black indicate that, although carbon black alone is not carcinogenic, solvent (benzene) extracts of carbon black are capable of inducing skin tumors following dermal exposure. Animal studies specifically examining the potential toxicity/carcinogenicity of printing inks are few. In a 1929 German study (Steinbruck, 1929), printing ink was applied to the dorsal skin of mice; 5 of 16 animals developed lesions described as "carcinomas." In a later study, CB mice were injected subcutaneously at weekly intervals for 15 - 22 weeks with 0.2 ml volumes of 22 printing inks, some of which were black inks; no significant tumor response was reported (Carter *et al.,* 1969).

Mutagenicity

There is no information in the published literature on the genetic toxicity of black newsprint inks, although data are available for their major constituents. Carbon black particles may contain varying amounts of PAHs, particularly nitropyrenes, adsorbed onto their surface. Agurell (1983) found benzene extracts of an unspecified carbon black to be mutagenic in TA98 and TA100 strains of *Salmonella* (with S9 activation); Kirwin *et al.* (1981), however, found that a sample of oil furnace carbon black, extracted with benzene, did not induce gene mutations in any of 5 strains of *S. typhimirium* or in mouse lymphoma L5178Y cells, either with or without S9 activation. The PAH content of this sample was determined to be 294 ppm. Extracts of photocopier toner (Lofroth *et al.,* 1980; Rosenkranz *et al.,* 1980) were found to be mutagenic, presumably due to nitropyrenes present as impurities in the carbon black colorant.

Numerous studies have been performed on the correlation between mutagenicity, PAH content, and carcinogenicity of mineral oils (IARC, 1984). In one report (Hermann *et al.,* 1980), various grades of mineral oils were either extracted with DMSO or dispersed in Tween 80, then tested for mutagenicity in *Salmonella* strain TA98; petroleum distillates, as well as refined samples, were mutagenic in the presence of S9, although the activities of the refined samples were significantly less than those of the unrefined samples. A medicinal grade oil was not mutagenic under the same test conditions. A good correlation was observed between mutagenic activity and PAH content of the oils; this correlation also was observed in another study of mineral oils, in which, in addition, mutagenicity and PAH content were found to be predictive of skin tumorigenicity in C3H mice (Roy *et al.,* 1988).

Study Rationale and Design

Black newsprint inks were nominated for carcinogenicity evaluation by NIOSH because of epidemiologic and experimental evidence of carcinogenicity in the newspaper printing environment, and because of the extensive potential of newsprint ink exposure to both workers and the public. Technological innovations have led to reductions in some occupational exposures, but ink formulations have not changed substantially and dermal exposure remains widespread. Lubricating press oils and newspaper fiber dust also may contribute to the observed cancer problems in the newspaper-printing environment; however, most interest has focused on newsprint inks and their component ingredients. Thus, a second objective of the proposed studies was to determine which major ingredient of the inks constitutes the greatest carcinogenic hazard. The dermal route of administration for various inks and mineral oil preparations was selected for this study, as it represents the major route of exposure in the workplace and to the public. The studies were carried out with F344/N rats and C3H mice of both sexes; C3H mice were used rather than the $B6C3F_1$ strain because of the extensive experience of the nominating agency (NIOSH) with this strain of mice in dermal studies. This report describes the results of genetic toxicity evaluations and of 30-day and 13-week *in vivo* studies that were performed to evaluate the toxicity of the inks and their component ingredients, and to provide a basis for selection of treatments for longer-term carcinogenicity studies.

MATERIALS AND METHODS

Procurement and Characterization of Samples

Black newsprint inks used in these studies were obtained as coded samples from Mr. Paul Volpe of the National Association of Printing Ink Manufacturers, Inc. (Harrison, NY). Four lots of both letterpress (NAPIM: A-1001, B-1002, C-1003, and D-1004) and offset (NAPIM: E-2001, F-2002, G-2003, and H-2004) ink types, representing a batch from each of the 4 major U.S. manufacturers, were supplied; composite lots of letterpress and offset ink were subsequently prepared by mixing the 4 separate lots of each type. Chemical characterization of each lot was performed by Midwest Research Institute (Kansas City, MO). Individual lots were analyzed by ultraviolet/visible and nuclear magnetic resonance spectroscopy; thin layer, high performance liquid and gas chromatography; and elemental analysis. Results indicated that the samples consisted of a heterogenous mixture of primarily aliphatic hydrocarbons, consistent with the composition of newsprint inks; carbon black concentrations likewise were compatible with expected values. Periodic elemental and turbidimetric analyses through the course of the study indicated that the composition of the bulk chemicals remained unchanged during the toxicity study. Samples of the letterpress and offset ink composite mixtures were further analyzed for 26 selected polycyclic aromatic hydrocarbons (PAHs) by high resolution gas chromatography/mass spectrometry selected ion monitoring. The letterpress ink composite was found to contain 22 of the selected PAHs with a cumulative concentration of 206 ppm; the offset ink mixture contained 21 of the selected and 4 additional PAHs with a cumulative concentration of 105 ppm. Specific analytes and their concentration in the ink samples are given in Appendix E.

Printing ink mineral oil (treated 750 oil, assigned lot No. 92686) was obtained from the National Association of Printing Ink Manufacturers, Inc. USP grade mineral oil (lot No. 6-0758) was supplied by Ashland Chemical Co. (Kansas City, KS). Both lots were chemically characterized by Midwest Research Institute. The densities of the USP mineral oil and the printing ink mineral oil, at approximately 23°C, were determined to be 0.845 and 0.924 g/ml, respectively. Mineral oils that have a density in the range of 0.83 - 0.86 are described as "light" oils, while those within a density range of 0.88 - 0.91 are referred to as "heavy" oils. Thus, the USP mineral oil used in this study is a light oil, while the printing ink mineral oil is a heavy oil, although the density of the latter falls slightly out of the usual density range.

Spectroscopic results for both were consistent with the composition of mineral oil. Gas chromatography of the USP mineral oil indicated the sample consisted of a mixture of aliphatic hydrocarbons with carbon numbers between C_{12} - C_{28}; a single broad peak, without resolution of individual components, was observed for the printing ink mineral oil sample. Periodic chemical reanalysis of the mineral oils through the course of the toxicity study was performed by gas chromatographic impurity profile comparison to reference standards. The 2 lots of mineral oil were further analyzed by high performance liquid chromatography for the presence of 26 selected PAHs. USP mineral oil contained no detectable aromatic hydrocarbons, while printing ink mineral oil contained 18 of the selected and 4 additional PAHs, with a cumulative concentration of 208 ppm (Appendix E). If the PAH levels in the printing ink mineral oil are compared to the levels in the

inks it would appear that the PAHs found in the inks come primarily from the mineral oil in the formulations.

Dose analyses and homogeneity studies of the ink-oil mixtures used in the repeated dose studies were performed periodically through the course of the toxicity study by ultraviolet spectroscopy (offset ink formulations) or by a gravimetric method (letterpress ink formulations). These were found to be within ± 10% of theoretical values.

30-Day Study Design

A 30-day study was performed to determine appropriate application techniques of the black newsprint inks and to describe any local or systemic toxicity following repeated dermal exposure to letterpress or offset newsprint inks. Groups of 5 rats and mice per sex were given a single daily dermal application of test chemical, 5 days a week for 30 days (a total of 21 - 22 applications). Dose delivery volume was 100 µl for mice and 250 µl for rats, applied to the shaved skin of the interscapular area. Hair was removed with electric clippers immediately prior to the first dose administration and twice weekly thereafter, prior to dosing.

Letterpress and offset newsprint inks used in the 30-day study were the composite mixtures of the 4 batches of each type. Undiluted ink mixtures as well as dilutions prepared with USP grade mineral oil were tested; dose groups for each type of ink were neat chemical (undiluted ink) and 3:1 (ink:mineral oil), 1:1, and 1:3 dilutions. Controls were exposed to printing ink mineral oil. Study data collected included body weights, morbidity/mortality, clinical observations for toxicity, and gross lesions at the site of application. The area of skin that encompassed the dose application site was excised and preserved in 10% neutral buffered formalin. Gross examination of the internal organs was not performed, nor was histopathologic examination of the skin performed. Additional details concerning study design and performance are given in Table 1.

13-Week Study Design

Groups of 10 rats and 10 mice of each sex were given dermal applications of neat letterpress or offset newsprint inks, or of mineral oil vehicles, to the clipped skin of the interscapular area. Based on methodological findings of the 30-day study, 20 µl (mice) or 50 µl (rats) volumes were applied at twice-weekly intervals. Hair was removed with electric clippers immediately prior to the first dose administration and twice weekly thereafter, prior to dosing.

Rats of each sex were divided into 5 treatment groups: letterpress ink mixture, offset ink mixture, USP grade mineral oil, printing ink mineral oil, and clipped, untreated controls. Mice of each sex were treated with each of the 4 individual batches of both letterpress inks (lots A-D) and offset inks (lots E-H), as well as their mixtures, and with USP and printing-ink mineral oils. A clipped, untreated group served as the control for a total of 13 treatment groups per sex.

Blood samples were collected at study termination and the sera analyzed for viral titers from 5 clipped, untreated control animals per sex and species. Data from 5 viral screens performed in rats and 12 viral screens performed in mice (Boorman *et al.,* 1986; Rao *et al.,* 1989) showed no positive antibody titers for these viruses.

Hematology studies were performed on rats and mice at study termination. The animals were anesthetized with a mixture of carbon dioxide (70%) and oxygen (30%) and bled from the retroorbital sinus. Samples (~0.5 ml) were collected in Microtainers® (Becton-Dickinson, Rutherford, NJ) containing dry potassium EDTA. Automated hematologic analyses were performed using an Ortho ELT-8 hematology system (Ortho Diagnostic Systems, Inc., Westwood, NJ). The following variables were measured (or calculated): erythrocytes, leukocytes, and platelet counts; hemoglobin (HGB) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Smears of peripheral blood were stained with Wright's stain and examined microscopically. Leukocyte differentials were determined on 100 cells and absolute counts of individual leukocytes were calculated based on the total leukocyte count and the relative number. Reticulocytes were stained by mixing equal volumes of whole blood with new methylene blue stain for 20 minutes. Relative numbers of reticulocytes, determined by microscopic examination of approximately 1000 erythrocytes, were converted to absolute counts based on the total erythrocyte count. Methemoglobin concentrations were measured by the spectrophotometric method of Evelyn and Malloy (1938).

Animals surviving to the end of the studies were killed with carbon dioxide, and complete necropsies were performed on all animals. Organs and tissues were examined for gross lesions. Liver, right kidney, heart, and lungs were weighed to the nearest 10 mg; right testis and thymus were weighed to the nearest 1 mg. Tissues were preserved in 10% neutral buffered formalin. Skin from the application site was excised and orientation clearly indicated before immersion in fixative. Untreated control skin was taken from the inguinal area. Tissues for microscopic evaluation were dehydrated and embedded in paraffin, sectioned at approximately 5 microns, and stained with hematoxylin and eosin. A complete histopathologic examination of all protocol-required tissues was performed on all animals (Table 1).

Upon completion of the histologic evaluation by the laboratory pathologist, 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, and the results were reviewed and evaluated in a NTP Pathology Review. The final diagnoses represent a consensus of contractor and reviewing pathologists. Additional details of study design, clinical examinations, and pathology procedures are outlined in Table 1.

Reproductive Toxicity

Sperm morphology and vaginal cytology evaluations were performed on rats from the 13-week study treated with letterpress ink mixture, offset ink mixture, and printing ink mineral oil, in addition to clipped, untreated controls. To screen for potential reproductive toxicity, epididymal sperm motility was evaluated at necropsy, and vaginal cytology was evaluated on animals during the 2 weeks just preceding necropsy, using procedures outlined by Morrissey *et al.* (1988). For the 12 days prior to sacrifice, females were subject to vaginal lavage with saline. The aspirated cells were air-dried onto slides, stained with Toluidine Blue O, and cover-slipped. The relative preponderance of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were used to identify the stages of the estrual cycle.

Sperm motility was evaluated at necropsy as follows: The left epididymis was removed and quickly weighed; the cauda epididymis was removed at the junction of the vas deferens and the corpus epididymis, then weighed. Warm (37°C) Tyrodes buffer (mice) or test yolk buffer (rats) was applied to 2 pre-warmed slides and a small cut made in the distal cauda epididymis. The sperm that extruded from the epididymis were dispersed throughout the solution, cover-slipped, and counted. Two independent observers counted the number of moving and non-moving sperm in 5 fields of 30 sperm or less per field. After sperm sampling for motility evaluation, the cauda was placed in phosphate buffered saline (PBS) and gently chopped with a razor blade; the remaining clumps of tissue were removed and the solution was mixed gently and heat-fixed at 65°C. Sperm density subsequently was determined using a hemocytometer.

To quantify spermatogenesis, the left testis was weighed, frozen and stored. After thawing, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the testis in PBS containing 10% DMSO. Homogenization-resistant spermatid nuclei were enumerated using a hemocytometer; the data were expressed as spermatid heads per total testis, and per gram of testis.

Genetic Toxicology

Testing was performed as reported by Ames *et al.* (1975), with modifications as listed below and described in greater detail in Haworth *et al.* (1983). The chemical was incubated with the *Salmonella typhimurium* tester strains, TA100 and TA98, either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37°C, top agar supplemented with L-histidine and D-biotin was added, and the contents of the tubes were mixed and poured on to the surfaces of minimal glucose agar plates. Incubation continued for an additional 48 hours.

Each test consisted of triplicate plates of concurrent positive and negative controls, and of at least 5 doses of test chemical. The high dose was limited by toxicity or solubility but did not exceed 10 mg/plate. All negative assays were repeated, and all positive assays were repeated under the conditions which elicited the positive response. A positive response is defined in this assay as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants which was not dose-related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment.

Statistical Methods

Analysis of Continuous Variables

Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedure of Dunnett (1955). Hematology data, which typically has skewed distributions, was analyzed using the nonparametric multiple comparisons method of Dunn (1964). 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.

Analysis of Vaginal Cytology Data

Since the data are ratios (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 the simultaneous equality of measurements across dose levels.

Quality Assurance

The studies of black newsprint ink were performed in compliance with FDA Good Laboratory Practices regulations (21 CFR 58). The Quality Assurance Unit of Battelle Columbus 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.

TABLE 1 Experimental Design and Materials and Methods in the 30-Day and 13-Week Dermal Studies of Black Newsprint Inks

Study Laboratory	Battelle Columbus Laboratories, Columbus OH
Study Dates	30-Day Dermal Studies: December 1-31, 1987 13-Week Dermal Studies: May 24 - September 16, 1988
Strain and Species	F344/N rats; C3H mice
Animal Source	30-Day Studies, Rats - Taconic Farms (Germantown, NY) 30-Day Studies, Mice - Frederick Cancer Research Facility (Frederick, MD) 13-Week Studies, Rats - Simonsen Laboratories (Gilroy, CA) 13-Week Studies, Mice - Charles River Laboratories (Portage, MI)
Chemical Source	National Association of Printing Ink Manufacturers, Inc. (Harrison, NY)
Size of Study Groups	30-Day Studies: 5 males and 5 females of each species, housed individually. 13-Week Studies: 10 males and 10 females of each species, housed individually.
Doses	30-Day Studies, Rats and Mice: Letterpress ink mixture: neat and 3:1, 1:1, and 1:3 dilutions with USP grade mineral oil; Offset ink mixture: neat and 3:1, 1:1, and 1:3 dilutions with USP grade mineral oil; Vehicle control: printing ink mineral oil. 13-Week Studies, Rats: letterpress ink mixture, offset ink mixture, printing ink mineral oil, USP mineral oil, and clipped, untreated control. 13-Week Studies, Mice: letterpress ink (4 individual lots and composite mixture), offset ink (4 individual lots and composite mixture), printing ink mineral oil, USP mineral oil, and clipped, untreated control.
Method of Animal Distribution	Animals distributed to weight classes, then assigned to groups by computer-generated random numbers.
Diet	NIH-07; available *ad libitum*
Animal Room Environment	Temp.: 72 ± 3°F; relative humidity: 50 ± 15%; 12 h fluorescent light/day
Time Held Before Study	11-12 days
Age When Placed on Study	7-8 wks
Duration of Dosing	30-Day Studies: 1 x d, 5 d/wk, for 21 doses over 28 days (mice - 29 days) 13-Week Studies: 2 x wk for 13 weeks
Age at Study Termination	30-Day Studies: 11 wks 13-Week Studies: 20-21 wks
Type and Frequency of Observation	30-Day Studies: Observed 2 x d for mortality/moribundity; 1 x d for clinical signs of toxicity; weighed before start of study, 1 x wk thereafter, and at study termination. 13-Week Studies: Observed 2 x d for mortality/moribundity; 1 x w for clinical signs of toxicity; weighed before start of study, 1 x wk thereafter, and at study termination.
Necropsy and Histologic Examinations (13-week studies)	Complete necropsy and histopathologic examination were performed on each animal. Skin tissues evaluated included: site-of-application (gross lesion), site-of-application (no gross lesion), and untreated skin (inguinal area). Non-cutaneous tissues examined included: gross lesions and tissue masses (if any), adrenals, brain (3 sections), esophagus, eyes (if grossly abnormal), femur (with bone marrow), gallbladder (mice), heart, intestines (duodenum, jejunum, ileum, cecum, colon, rectum), kidneys, liver, lungs and mainstem bronchi, lymph nodes (mesenteric and mediastinal), mammary gland, muscle (thigh), nasal cavity and turbinates (3 sections), ovaries/uterus/vagina (females) or testis/epididymis/prostate and seminal vesicle (males), pancreas, pharynx (if grossly abnormal), pituitary, preputial or clitoral glands, salivary gland, spinal cord and sciatic nerve (if neurological signs present), spleen, stomach (forestomach and glandular stomach), thymus, thyroid and parathyroid, trachea, and urinary bladder.

RESULTS

30-Day Studies in F344/N Rats

Thirty-day studies were conducted to determine appropriate techniques for applying test inks and mineral oils at the site of application and to determine the appropriate frequency of application as well as the irritation potential of black newsprint inks. The use of a positive displacement pipette was found to be most effective in giving consistently accurate aspiration and deposition of chemical at the test site. Daily applications of the test inks and the relatively large dose volume (250 µl) resulted in accumulation of inks at the dermal test site. Electric clippers were found to be the method of choice for removal of this ink build-up. As a result of the grooming activity of the animals and the large amounts of chemical applied, ink was spread over the body, giving the fur a gray-black discoloration and a greasy, ungroomed appearance. Ink also was found on the paws and around the face, suggesting that some oral ingestion of the inks had occurred.

Mortality and body weight data are summarized in Table 2. There were no unscheduled deaths during this study. In male rats, there were no marked effects on mean body weight or body weight gain by treatment with letterpress or offset ink formulations, relative to the control group treated group. Decreases in mean final body weights and body weight gains were observed in female rats given neat and 3:1 dilution formulations of letterpress ink, compared to the mineral oil control group. No definitive changes in body weight values were observed in female rats treated with offset ink formulations. Treatment-related clinical findings for rats of both sexes were limited to gray-black discoloration of the hair coat in most ink-treated animals. No gross lesions at the site of application were noted during the study or at necropsy.

13-Week Studies in F344/N Rats

In order to prevent the accumulation of test inks and mineral oils at the application site that occurred in the 30-day study, the volume of chemical administered per dose in the 13-week study was reduced from 250 µl to 50 µl, and the frequency of application was reduced from daily to twice weekly. All rats in all dose groups survived until scheduled termination (Table 3). Group mean body weight data are presented in Table 3 and shown graphically in Figure 1. In male rats, there were slight but not statistically significant depressions in mean final body weights and weight gains of all treatment groups relative to clipped, untreated controls. Greater depressions in mean body weights and body weight gains, compared to controls, were observed in some female rat groups; this effect was significant statistically in females exposed to the letterpress ink mixture and to printing ink mineral oil. No clinical observations could be attributed to chemical administration, except for the black discoloration at the application site in all rats receiving the letterpress or offset ink mixtures. There was no discoloration of the mouth that would indicate oral ingestion of ink.

At necropsy, no apparent chemical-related gross lesions were observed in rats of either sex. Both male and female rats exposed to USP mineral oil showed group mean absolute liver weights and liver-to-body-weight ratios that were elevated relative to their respective controls (Table 4). Absolute and relative kidney weights also were increased in both sexes given USP mineral oil (Table 4). Female rats administered letterpress ink mixture exhibited group mean absolute heart and

lung weights which were decreased relative to controls. Complete organ weight data are presented in Appendix A.

TABLE 2 Survival and Weight Gain of F344/N Rats in the 30-Day Dermal Studies of Black Newsprint Inks

Treatment Groups	Survival[a]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[c]
		Initial	Final	Change[b]	
MALE					
Mineral oil[d]	5/5	150	219	69	
Letterpress ink					
Neat	5/5	161	222	61	101
3:1[e]	5/5	156	214	58	98
1:1	5/5	155	232	77	106
1:3	5/5	155	246	91	112
Offset ink					
Neat	5/5	151	242	91	111
3:1	5/5	151	224	73	102
1:1	5/5	148	242	94	111
1:3	5/5	156	240	84	110
FEMALE					
Mineral oil	5/5	127	156	29	
Letterpress ink					
Neat	5/5	125	134	9	86
3:1	5/5	126	137	11	88
1:1	5/5	126	153	27	98
1:3	5/5	123	158	35	101
Offset ink					
Neat	5/5	123	147	24	94
3:1	5/5	126	145	19	93
1:1	5/5	126	148	22	95
1:3	5/5	127	166	39	106

a Number surviving to study termination / number of animals per group.
b Mean weight change of the animals in each dose group.
c (Dosed group mean / control group mean) x 100.
d Printing ink mineral oil.
e Parts ink:parts USP mineral oil; applies to all mixtures.

TABLE 3 Survival and Weight Gain of F344/N Rats in the 13-Week Dermal Studies of Black Newsprint Inks

Treatment Groups	Survival[a]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[c]
		Initial	Final	Change[b]	
MALE					
Clipped, untreated	10/10	134	335	201	
USP Mineral Oil	10/10	134	332	198	99
Printing Ink Mineral Oil	10/10	132	326	194	97
Letterpress Ink Mix	10/10	135	328	193	98
Offset Ink Mix	10/10	133	324	191	97
FEMALE					
Clipped, untreated	10/10	109	193	84	
USP Mineral Oil	10/10	109	191	82	99
Printing Ink Mineral Oil	10/10	109	184	75	95
Letterpress Ink Mix	10/10	109	176	67	91
Offset Ink Mix	10/10	108	185	77	96

a Number surviving to study termination / number of animals per group.
b Mean weight change of the animals in each dose group.
c (Dosed group mean / control group mean) x 100.

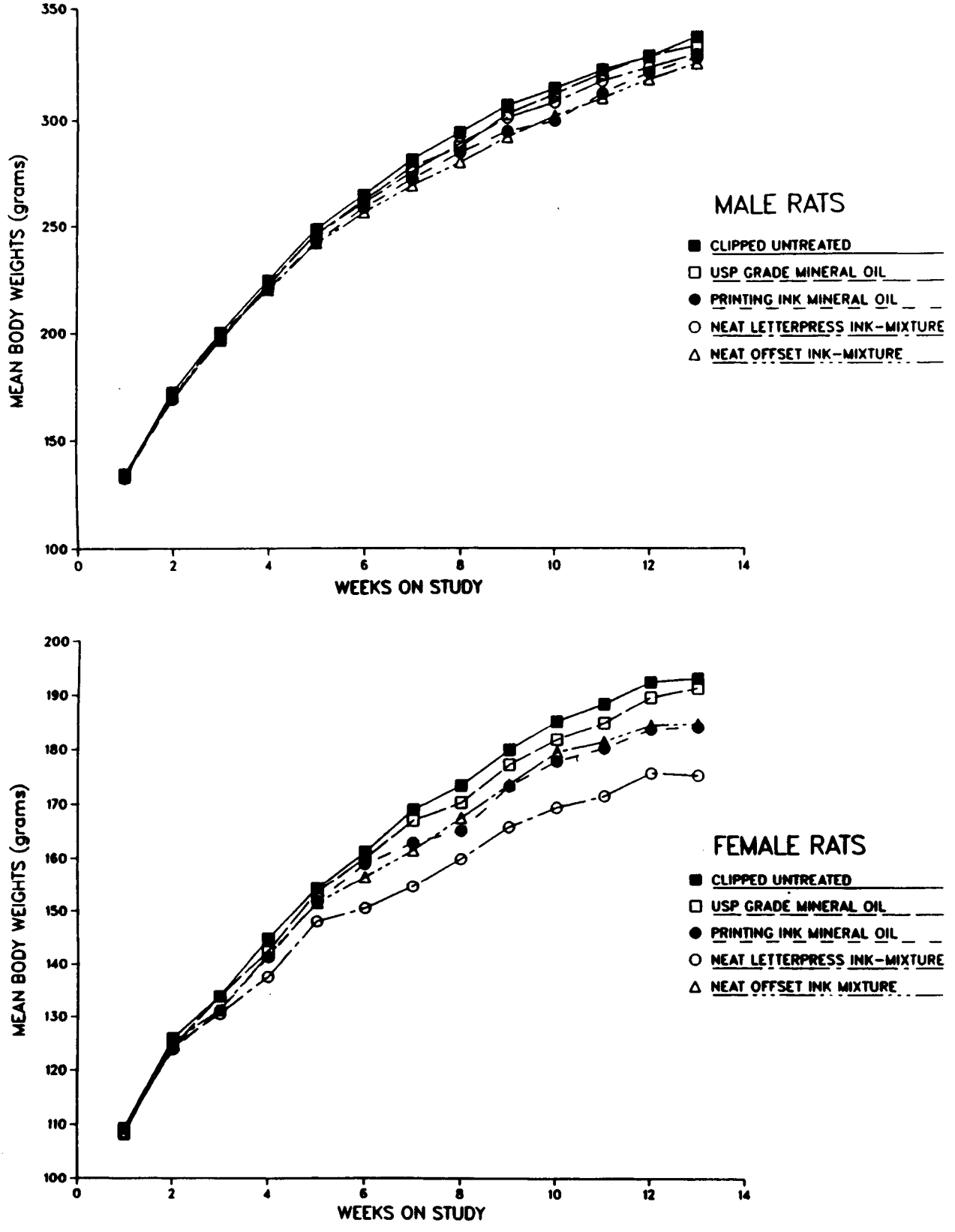

Figure 1 Growth Curves of F344/N Rats in the 13-Week Dermal Studies of Black Newsprint Inks and Mineral Oils

TABLE 4 Selected Organ Weights of F344/N Rats in the 13-Week Dermal Studies of Black Newsprint Inks[a]

Organ	Clipped Untreated	USP Mineral Oil	Printing Ink Mineral Oil	Letterpress Ink Mixture	Offset Ink Mixture
MALE					
Body Weight[b]	341.3 ± 6.3	336.1 ± 4.2	331.8 ± 6.0	331.9 ± 3.3	327.4 ± 5.3
Right Kidney					
Absolute[c]	1232 ± 29	1336 ± 25*	1231 ± 25	1213 ± 21	1223 ± 39
Relative[d]	3.62 ± 0.07	3.98 ± 0.07**	3.71 ± 0.05	3.66 ± 0.06	3.73 ± 0.08
Liver					
Absolute[e]	14.0 ± 0.4	16.0 ± 0.4**	14.6 ± 0.4	13.5 ± 0.3	13.8 ± 0.5
Relative[f]	4.10 ± 0.09	4.77 ± 0.13**	4.40 ± 0.09	4.07 ± 0.09	4.20 ± 0.13
FEMALE					
Body Weight[b]	194.6 ± 2.5	192.3 ± 1.8	183.2 ± 1.8	176.1 ± 3.0	185.4 ± 1.9
Right Kidney					
Absolute[c]	738 ± 10	825 ± 9**	713 ± 13	698 ± 17	738 ± 13
Relative[d]	3.82 ± 0.07	4.29 ± 0.05**	3.89 ± 0.05	3.96 ± 0.05	3.98 ± 0.06
Liver					
Absolute[e]	7.3 ± 0.2	8.3 ± 0.2**	7.1 ± 0.1	6.9 ± 0.2	7.1 ± 0.1
Relative[f]	3.73 ± 0.07	4.33 ± 0.09**	3.90 ± 0.06	3.89 ± 0.04	3.85 ± 0.07

a Groups of 10 animals each sex; all animals survived.
b Body weights are presented as mean ± standard error in grams.
c Absolute weights are presented as mean ± standard error in milligrams.
d Relative weights are presented as mean ± standard error in milligrams per gram of body weight.
e Absolute weights are presented as mean ± standard error in grams.
f Relative weights are presented as mean ± standard error in grams per gram of body weight x 100.
* Significantly different from control groups using Dunnett's Test. ($p \leq 0.05$)
** Significantly different from control groups using Dunnett's Test: ($p \leq 0.01$)

Exposure of rats to black newsprint inks or mineral oils produced no hematologic effects (Appendix B). There were no effects on reproductive parameters in either male or female rats in any treatment group (Appendix C).

Histopathologic examination revealed no lesions that were considered related to administration of black newsprint inks or mineral oils to rats. Black pigment was seen microscopically on the surface of the keratinized epithelium of the skin; the presence of this pigment was not considered a lesion, however, and it was not entered into histopathology data tables.

30-Day Studies in C3H Mice

As in the 30-day studies with rats, a positive displacement pipette was most effective in consistent aspiration and application of the chemicals. The repeated daily applications of inks resulted in an accumulation of the chemical at the site; electric clippers were required to remove this build-up. It was not readily apparent whether test inks were spread over the body by grooming activity, as was seen in the rats, due to the dark fur coat of the C3H mice; however, a greasy and ruffled appearance of the fur was noted, suggesting that spreading of the test chemicals had occurred.

Survival and body weight data are summarized in Table 5. All animals survived until scheduled termination. Final group mean body weights for male and female mice treated with both letterpress and offset ink formulations were similar to the printing ink mineral oil controls. Group mean body weight gains of males in all treatment groups tended to be slightly increased more than that of the mineral oil control group. Group mean body-weight gains of females generally were similar to the mineral oil control group; decreased values in some groups were not considered biologically significant. The site of application was normal in appearance in mice of both sexes treated with either printing ink mineral oil or with neat and diluted offset inks. Scaliness at the site of application was a treatment-related observation noted at study termination in both sexes administered letterpress ink. One or more mice per treatment group were affected with all letterpress ink formulations except the female letterpress 1:3 dilution group. There was no redness or other evidence to indicate that the ink caused dermal irritation.

TABLE 5 Survival and Weight Gain of C3H Mice in the 30-Day Dermal Studies of Black Newsprint Inks

Treatment Groups	Survival[a]	Mean Body Weight (grams) Initial	Final	Change[b]	Final Weight Relative to Controls (percent)[c]
MALE					
Mineral oil[d]	5/5	24.3	27.8	3.5	
Letterpress ink					
Neat	5/5	24.3	28.8	4.5	104
3:1[e]	5/5	24.0	28.7	4.7	103
1:1	5/5	24.0	27.8	3.8	100
1:3	5/5	23.9	28.9	5.0	104
Offset ink					
Neat	5/5	24.2	28.0	3.8	101
3:1	5/5	24.6	29.8	5.2	107
1:1	5/5	23.5	29.0	5.5	104
1:3	5/5	24.4	28.1	3.7	101
FEMALE					
Mineral oil	5/5	19.8	24.7	4.9	
Letterpress ink					
Neat	5/5	21.4	26.0	4.6	105
3:1	5/5	20.0	24.7	4.7	100
1:1	5/5	20.2	24.6	4.4	99
1:3	5/5	20.7	24.6	3.9	99
Offset ink					
Neat	5/5	20.0	24.7	4.7	100
3:1	5/5	20.1	25.1	5.0	102
1:1	5/5	20.1	23.9	3.8	97
1:3	5/5	19.7	24.2	4.5	98

[a]Number surviving to study termination / number of animals per group.
[b]Mean weight change of the animals in each dose group
[c](Dosed group mean / control group mean) x 100
[d]Printing ink mineral oil
[e]Parts ink:parts USP grade mineral oil; applies to all mixtures.

13-Week Studies in C3H Mice

All male mice survived until scheduled study termination (Table 6). One female mouse in the offset ink, lot E group was killed due to moribundity on study day 71; all other female mice survived to study termination. Group mean body weight data are presented in Table 6 and shown graphically in Figure 2. Over the course of the study, the male and female treatment groups showed group mean body weights and body weight gains that were similar to their respective clipped, untreated control groups.

TABLE 6 Survival and Weight Gain of C3H Mice in the 13-Week Dermal Studies of Black Newsprint Inks

Treatment Groups	Survival[a]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[c]
		Initial	Final	Change[b]	
MALE					
Clipped, untreated	10/10	23.3	32.7	9.4	
USP mineral oil	10/10	23.9	33.5	9.6	102
Printing ink mineral oil	10/10	23.6	34.2	10.6	105
Letterpress lot A	10/10	23.4	34.0	10.6	104
Letterpress lot B	10/10	23.4	33.6	10.2	103
Letterpress lot C	10/10	23.5	34.1	10.6	104
Letterpress lot D	10/10	24.0	34.4	10.4	105
Letterpress mix	10/10	21.5	33.6	12.1	103
Offset lot E	10/10	23.1	33.6	10.5	103
Offset lot F	10/10	22.7	32.8	10.1	100
Offset lot G	10/10	22.3	32.6	10.3	99
Offset lot H	10/10	22.4	32.9	10.5	101
Offset mix	10/10	23.4	32.9	9.5	101
FEMALE					
Clipped, untreated	10/10	19.5	29.7	10.2	
USP mineral oil	10/10	19.8	30.4	10.6	102
Printing ink mineral oil	10/10	20.2	31.9	11.7	107
Letterpress lot A	10/10	19.6	30.2	10.6	102
Letterpress lot B	10/10	20.0	29.6	9.6	100
Letterpress lot C	10/10	20.0	30.2	10.2	102
Letterpress lot D	10/10	20.0	30.6	10.6	103
Letterpress mix	10/10	20.0	29.9	9.9	101
Offset lot E	9/10	20.1	30.0	9.9	101
Offset lot F	10/10	19.8	30.1	10.3	101
Offset lot G	10/10	19.7	29.9	10.2	101
Offset lot H	10/10	20.0	29.9	9.9	101
Offset mix	10/10	19.5	29.6	10.1	100

a Number surviving to study termination / number of animals per group.

b Mean weight change of the animals in each dose group.

c [Dosed group mean / control group mean] x 100.

A black staining of the skin occurred at the site of application in all mouse groups administered letterpress or offset inks. This discoloration was limited to the test site; there was no indication of spreading of the ink away from this site. Signs of dermal toxicity at the site of application were observed in male and female mice administered USP mineral oil and letterpress ink, lot C. Treatment with USP mineral oil resulted in scaliness of the skin at the application site in all mice, which was apparent at study day 24 and was followed by dermal irritation in all males and 8 of 10 females by study day 38. In mice treated with letterpress ink, lot C, scaliness developed in 8 of 10 males and 6 of 10 females by study day 38, and was followed by signs of test site irritation in 7 males and 3 females at study days 52 - 66. One of the males in this treatment group subsequently developed an ulcer at the application site by study day 73. One male mouse exposed to the letterpress ink mixture had similar scaliness and irritation at the test site. No other clinical observations were attributed to administration of newsprint inks or mineral oils.

A black staining of the skin occurred at the site of application in all mouse groups administered letterpress or offset inks. This discoloration was limited to the test site; there was no indication of spreading of the ink away from this site. Signs of dermal toxicity at the site of application were observed in male and female mice administered USP mineral oil and letterpress ink, lot C.

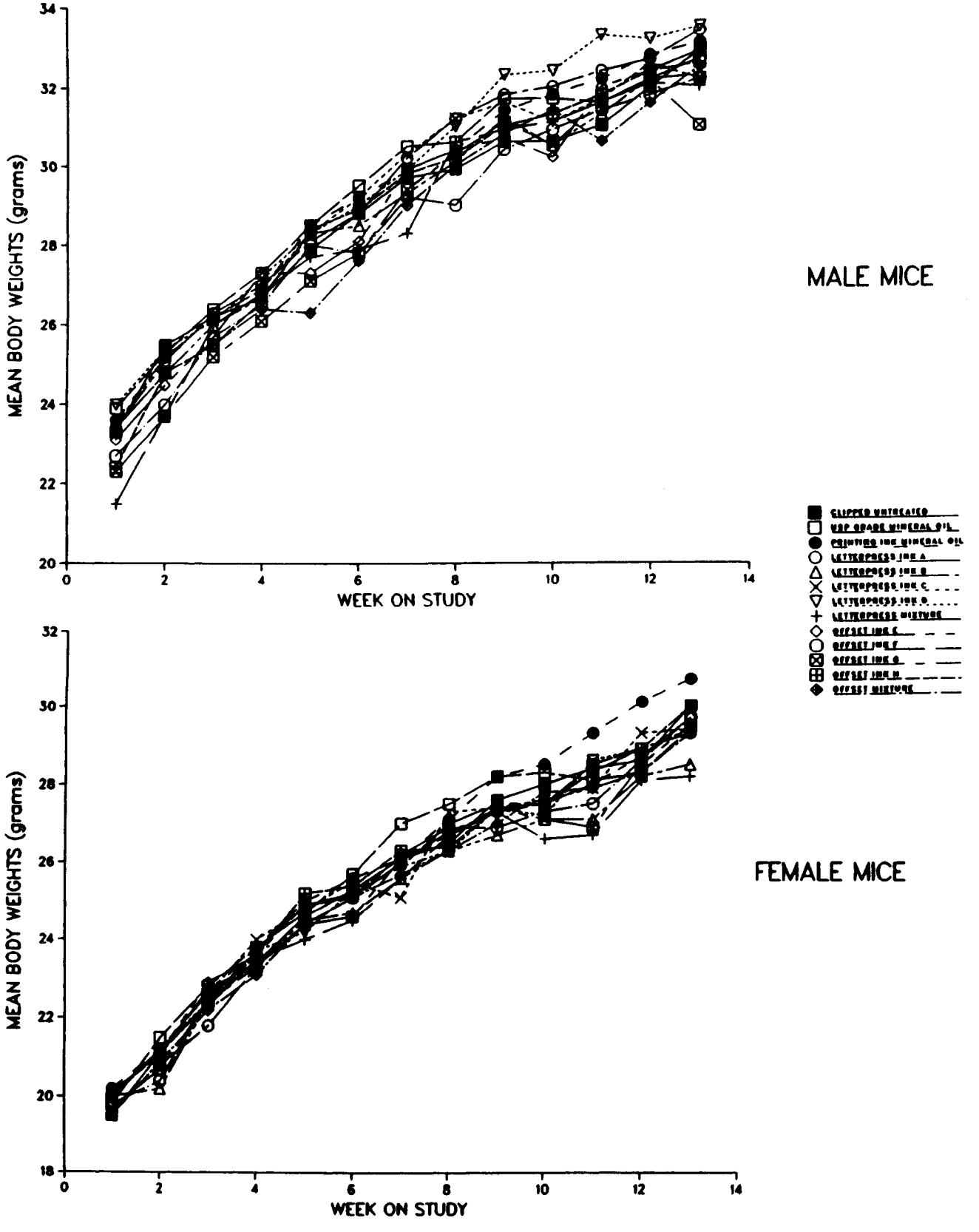

Figure 2 Growth Curves of C3H Mice In the 13-Week Dermal Studies of Black Newsprint Inks and Mineral Oils

Treatment with USP mineral oil resulted in scaliness of the skin at the application site in all mice, which was apparent at study day 24 and was followed by dermal irritation in all males and 8 of 10 females by study day 38. In mice treated with letterpress ink, lot C, scaliness developed in 8 of 10 males and 6 of 10 females by study day 38, and was followed by signs of test site irritation in 7 males and 3 females at study days 52 - 66. One of the males in this treatment group subsequently developed an ulcer at the application site by study day 73. One male mouse exposed to the letterpress ink mixture had similar scaliness and irritation at the test site. No other clinical observations were attributed to administration of newsprint inks or mineral oils.

At necropsy, treatment-related gross lesions in mice were limited to the skin at the site of chemical application. Gross lesions corresponded to the clinical observations described above and consisted of dermal scaliness and/or irritation. This was noted in most mice of both sexes treated with USP mineral oil, several mice of both sexes treated with letterpress ink-lot C, and in a single male mouse treated with letterpress ink mixture. Incidences of these gross findings are tabulated in Table 8. Black discoloration of the treated skin was observed at the test site of the majority of ink-treated mice of both sexes. In 2 female mice of the clipped, untreated control group, skin from the SOA had minimal acanthosis and mild inflammation; the inflammation in these animals, however, consisted of focal perifollicular infiltrates, in contrast to the more diffuse infiltration observed to be associated with administration of the various test compounds.

Increases in absolute and/or relative liver weight were observed in most treatment groups (Table 7). The most significant increases occurred in male and female mice treated with letterpress ink-lot C and in male mice exposed to printing ink mineral oil and letterpress ink mixture. There were sporadic, statistically significant increases in absolute weights of the kidney, heart, and thymus throughout the various treatment groups. These variations in organ weights were not thought to be treatment-related. No organ-weight changes were observed in the lung or testis. Complete organ-weight data are presented in Appendix A.

Statistical analyses indicated that the mean values of some hematologic variables from female treatment groups were significantly changed from their respective controls; however, the changes were minimal and not considered biologically significant (Appendix B). Complete histopathologic examination was performed on all mice in all treatment groups. Treatment-related effects were limited to the dermal site of application, as described below. Although liver weights were elevated in many treatment groups, no corresponding histologic lesion in this organ was observed.

In general, treatment-related skin lesions at the site of application (SOA) consisted primarily of epidermal acanthosis (hyperplasia) and dermal inflammation (Table 8). These lesions were present in sections with no apparent gross lesion as well as in sections of SOA's with grossly visible lesions. Acanthosis was minimal to moderate in severity and was characterized by an increased thickness of the epidermis due to an increase in the number of epithelial cell layers (Plates 2-4). Acanthosis was multifocal to diffuse, depending on severity. Increased thickness of the superficial keratin layer was variably associated with the acanthotic lesion. Dermal inflammation was characterized as chronic, active in nature, and minimal to moderate in severity, and consisted of diffuse infiltration of leukocytes (neutrophils and lymphocytes predominately) (Plates 2-4).

TABLE 7 Liver Weights of C3H Mice in the 13-Week Dermal Studies of Black Newsprint Inks[a]

Treatment Group	Body weight	Absolute liver weight	Relative liver weight
MALE			
Clipped, untreated	32.7 ± 1.1	1.70 ± 0.05	5.20 ± 0.10
USP mineral oil	33.5 ± 0.7	1.87 ± 0.03*	5.58 ± 0.12
Printing ink mineral oil	34.2 ± 0.8	2.00 ± 0.04**	5.85 ± 0.13**
Letterpress ink			
lot A	34.0 ± 0.6	1.94 ± 0.06*	5.72 ± 0.14*
lot B	33.6 ± 0.6	1.89 ± 0.05	5.64 ± 0.15
lot C	34.1 ± 0.6	2.00 ± 0.05**	5.86 ± 0.12**
lot D	34.4 ± 0.9	1.85 ± 0.07	5.38 ± 0.19
mix	33.6 ± 0.8	2.02 ± 0.07**	6.01 ± 0.13**
Offset ink			
lot E	33.6 ± 0.8	1.91 ± 0.04*	5.68 ± 0.06*
lot F	32.8 ± 0.7	1.86 ± 0.06	5.67 ± 0.12*
lot G	32.6 ± 0.7	1.83 ± 0.06	5.61 ± 0.14*
lot H	32.9 ± 0.7	1.91 ± 0.05*	5.79 ± 0.11**
mix	32.9 ± 1.1	1.93 ± 0.06*	5.89 ± 0.10**
FEMALE			
Clipped, untreated	29.7 ± 1.1	1.59 ± 0.07	5.36 ± 0.13
USP mineral oil	30.4 ± 0.7	1.76 ± 0.03	5.82 ± 0.12
Printing ink mineral oil	31.9 ± 1.0	1.84 ± 0.05**	5.81 ± 0.17
Letterpress ink			
lot A	30.2 ± 1.1	1.77 ± 0.07	5.88 ± 0.13
lot B	29.6 ± 0.5	1.76 ± 0.04	5.97 ± 0.12*
lot C	30.2 ± 0.9	1.99 ± 0.04**	6.61 ± 0.13**
lot D	30.6 ± 1.0	1.79 ± 0.06*	5.87 ± 0.17
mix	29.9 ± 0.6	1.83 ± 0.04**	6.15 ± 0.18**
Offset ink			
lot E	30.0 ± 1.0	1.84 ± 0.1*	6.11 ± 0.28*
lot F	30.1 ± 0.7	1.76 ± 0.05	5.87 ± 0.13
lot G	29.9 ± 0.8	1.88 ± 0.07*	6.29 ± 0.18**
lot H	29.9 ± 0.4	1.73 ± 0.04	5.79 ± 0.17
mix	29.6 ± 0.6	1.78 ± 0.06	6.02 ± 0.18*

[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error); n=10.
* Significantly different from the clipped, untreated control group by Dunnett's test ($p \leq 0.05$).
** Significantly different from the clipped, untreated control group by Dunnett's test ($p \leq 0.01$).

As seen in Table 8, acanthosis and inflammation at the SOA occurred in all treatment groups. Although incidence and severity tended to be greater in the groups with gross lesions (i.e., USP mineral oil, lot C letterpress ink, letterpress ink mixture), microscopic lesions were present to a variable degree in the treated skin of all groups. Ulceration of the epidermis was present in a few mice, particularly in the USP mineral oil and lot C letterpress ink treatment groups of both sexes. Ulceration was minimal to mild in severity, consisting of focal, circumscribed areas of epidermal loss (Plates 3, 4). Slightly increased numbers of mast cells were a component of the dermal inflammation in many animals, but in 1 male and 1 female mouse treated with lot C letterpress ink, distinct focal accumulations of mast cells (diagnosed as mastocytosis) were seen. In 2 female mice of the clipped, untreated treatment group, skin from the SOA had minimal acanthosis and mild inflammation. Untreated skin from a site distant to the SOA (inguinal area) was normal in all mice.

TABLE 8 Incidence (Severity) of Lesions at the Site of Application (SOA) in the 13-Week Dermal Studies of Black Newsprint Inks in C3H Mice[a]

	TREATMENT GROUP												
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
MALES													
GROSS LESIONS[b]		10				7		1					
MICROSCOPIC:													
SOA With Gross Lesion													
Acanthosis	-	10 (2.3)	-	-	-	7 (3.0)	-	1 (3.0)	-	-	-	-	-
Inflammation	-	10 (2.0)	-	-	-	7 (2.6)	-	1 (2.0)	-	-	-	-	-
Ulceration	-	5 (1.2)	-	-	-	5 (1.4)	-	0	-	-	-	-	-
Mastocytosis	-	0	-	-	-	1 (1.0)	-	0	-	-	-	-	-
SOA Without Gross Lesion													
Acanthosis	0	8 (1.9)	3 (1.0)	9 (1.0)	10 (1.3)	7 (2.6)	7 (1.0)	9 (1.7)	7 (2.0)	8 (1.2)	10 (1.6)	9 (1.0)	9 (1.0)
Inflammation	0	8 (1.8)	3 (1.0)	4 (1.0)	7 (1.3)	7 (2.3)	6 (1.0)	8 (1.4)	6 (1.0)	7 (1.1)	10 (1.0)	5 (1.0)	5 (1.0)
Ulceration	0	2 (1.0)	0	0	0	0	0	0	0	1 (1.0)	0	0	0
FEMALES													
GROSS LESIONS[b]		8				3							
MICROSCOPIC:													
SOA With Gross Lesion													
Acanthosis	-	8 (2.6)	-	-	-	3 (2.3)	-		-	-	-	-	-
Inflammation	-	8 (2.0)	-	-	-	3 (2.0)	-		-	-	-	-	-
Ulceration	-	5 (1.0)	-	-	-	1 (1.0)	-		-	-	-	-	-
Mastocytosis	-	0	-	-	-	1 (4.0)	-		-	-	-	-	-
SOA Without Gross Lesion													
Acanthosis	2 (1.0)	6 (1.8)	3 (1.0)	6 (1.0)	9 (1.1)	6 (1.5)	5 (1.0)	10 (1.7)	10 (1.1)	10 (1.3)	9 (1.8)	10 (1.1)	10 (1.0)
Inflammation	2 (2.0)	6 (1.5)	3 (1.0)	8 (1.0)	10 (1.0)	6 (1.5)	5 (1.0)	10 (1.3)	10 (1.0)	10 (1.0)	9 (1.1)	10 (1.0)	10 (1.0)
Ulceration	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] Ten mice per group examined. Average severity score based on a scale of 1 to 4: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Scores are averages based on the number of animals with lesions.

[b] Scaliness and irritation

Key to Treatment Groups:

1 = clipped,untreated
2 = USP mineral oil
3 = printing ink mineral oil
4 = letterpress ink, lot A
5 = letterpress ink, lot B
6 = letterpress ink, lot C
7 = letterpress ink, lot D
8 = letterpress ink mixture
9 = offset ink, lot E
10 = offset ink, lot F
11 = offset ink, lot G
12 = offset ink, lot H
13 = offset ink mixture

Plates

Plate 1. Skin from the site of application of a clipped, untreated male mouse. 75X

Plate 2. Mild epidermal acanthosis and dermal inflammation at the site of application from a male mouse treated with USP mineral oil. 75X

Plate 3. Moderate acanthosis and inflammation with focal ulceration (U) at the site of application from a male mouse treated with USP mineral oil. 75X

Plate 4. Moderate acanthosis, ulceration, inflammation and associated surface ink pigment (P) at the site of application from a male mouse treated with letterpress black newsprint ink (Lot C). 75X

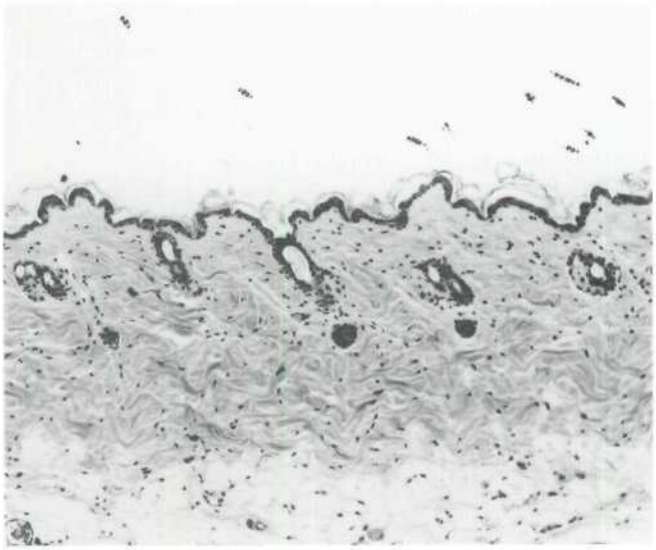

Plate 1

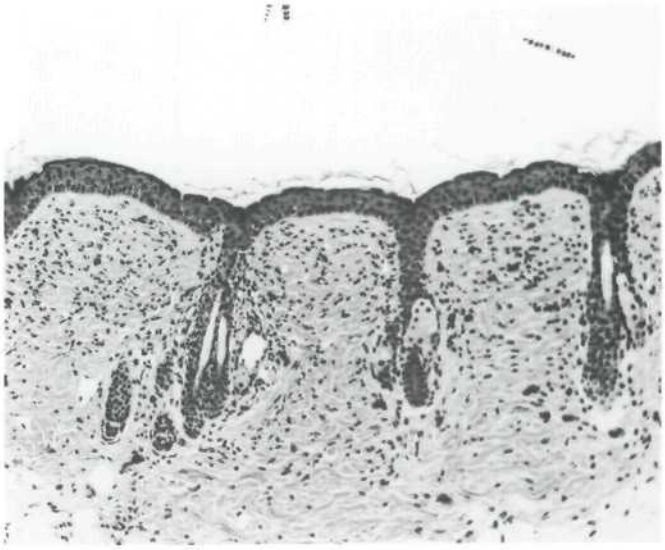

Plate 2

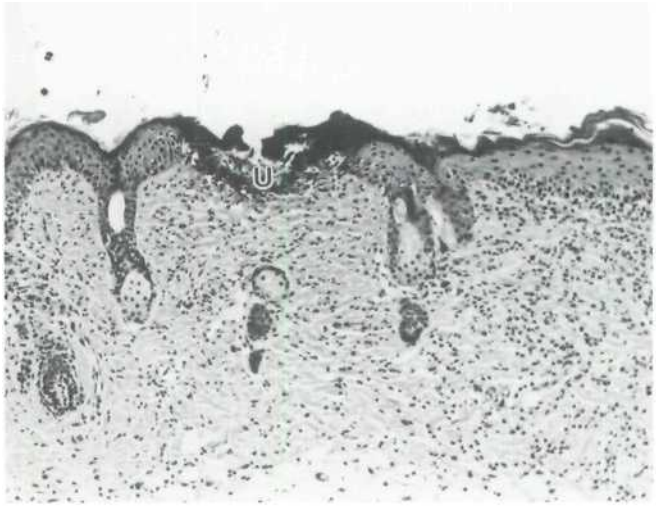

Plate 3

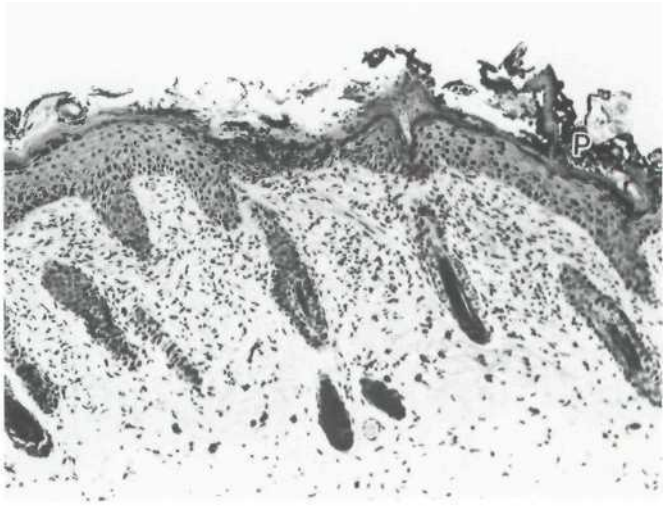

Plate 4

Genetic Toxicology

Letterpress and offset black newsprint ink composite mixtures were tested in a preincubation protocol at concentrations of 100-10,000 μg/plate for induction of gene mutations in *Salmonella typhimurium* strains TA100 and TA98 (Appendix D). Tests were conducted with and without Aroclor 1254-induced male Sprague-Dawley rat and Syrian hamster liver S9. Neither type of ink was mutagenic in the absence of S9 activation. In the presence of 30% hamster liver S9, positive responses were obtained for both the letterpress and offset inks in both strains of *Salmonella.* In the presence of rat liver S9, both inks were negative in strain TA100 and positive in strain TA98.

DISCUSSION

Black newsprint ink is a complex mixture of carbon black in a mineral oil vehicle with various other petroleum-based additives. Newsprint inks were selected for toxicity study because of widespread occupational and public exposure, primarily by the dermal route. The studies described in this report were conducted to develop specific application techniques and to characterize the toxicity of newsprint inks and two different grades of mineral oil by dermal exposure. In 30-day repeated dose studies, there was no evidence of systemic toxicity resulting from the topical application of neat or diluted inks or mineral oils; local toxicity was limited to scaliness at the site of application in some mice treated with letterpress ink formulations. The volume and frequency of application in these studies was excessive in that ink accumulated at the site of application, was spread over the body by grooming activity, and possibly was ingested. Therefore, the volume of chemical administered and the frequency of application was reduced in subsequent 13-week studies, to allow sufficient contact of the test chemicals with the skin but to minimize distribution to other locations.

In general, there were few indications of systemic toxicity in rats or mice resulting from 13-week dermal exposures to black newsprint inks or mineral oils. These results are not unexpected, since little percutaneous absorption of the long-chain and polycyclic hydrocarbons which comprise these compounds was anticipated. No mortality was observed that could be attributed to compound administration. Body-weight effects were limited to decreased mean final body weights and body-weight gains in female rats administered printing ink, mineral oil, and a letterpress ink mixture. Increases in absolute and/or relative liver weights were consistently seen in most treatment groups in mice; however, there was no corresponding histologic lesion associated with these effects. Microscopic changes were limited to the dermal site of application. Absence of significant hematologic and reproductive system effects is further evidence for the lack of systemic toxicity of these test chemicals following dermal exposure.

Local toxicity of newsprint inks and mineral oils in the 13-week studies was evidenced by clinical observations of scaliness and irritation and the histologic lesions of acanthosis (hyperplasia), ulceration, and inflammation at the site of application. Prolonged contact with petroleum oils results in a known occupational dermatosis characterized by erythema and dryness of the skin (Birmingham, 1988). This observation is attributed to the fact that liquid hydrocarbons are fat solvents and, when applied repetitively, result in the constant removal of skin surface lipids which leads to dehydration and cracking of the keratin layer. Acanthosis, or epidermal hyperplasia, was a treatment-related effect of all inks as well as of the two mineral oils tested in these studies; this lesion is a common response of the skin to a wide variety of chemical agents, including petroleum-derived hydrocarbons (Argyris, 1981). Attention has focused on polycyclic aromatic hydrocarbons (PAHs), known to be initiators of skin carcinogenesis in initiation/promotion protocols, or as complete carcinogens (DiGiovanni, 1989). However, aliphatic hydrocarbons may also cause acanthosis and ulceration of the skin, as reported in studies of refined petroleum oils (Doak *et al.,*1983; Horton *et al.,* 1957) and as seen in these studies by the finding of relatively severe skin irritant effects of PAH-free, USP-grade mineral oil. The finding of focal mast cell accumulations in 2 letterpress ink-treated mice is of interest in the light of reported mast cell tumors induced by

various skin carcinogenesis protocols (Miyakawa *et al.,* 1990; Farnoush and Mackenzie, 1984; Ohmori and Rivenson, 1981).

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In summary, results of these studies indicate that dermal administration of black newsprint inks and mineral oils produce local toxicity at the site of application in mice; toxic effects on the skin in this species are consistent with those of a primary cutaneous irritant. In rats, possible evidence for toxicity was limited to decreased body-weight gains in females treated with letterpress ink formulations. Due to the complex nature of these compounds, it is impossible to attribute these effects to any single constituent. Similarly, it is difficult to predict the carcinogenic potential of such mixtures, given the variable and frequently unknown chemical composition of petroleum derivatives, and the unpredictable manner in which so many potentially biologically active chemicals could interact as initiators, promoters, cocarcinogens, inhibitors, etc. However, given the weight of experimental evidence that petroleum-derived compounds are carcinogenic to mouse skin and the mutagenicity for *Salmonella* of the 2 composite mixtures, it is predictable that newsprint inks would be carcinogenic in a chronic bioassay. A previous NTP bioassay has demonstrated a positive skin tumor response following dermal exposure to a complex petroleum-based mixture containing aliphatic and aromatic hydrocarbons (NTP,1986), and the IARC (1984) has determined that there is sufficient evidence to consider untreated and mildly treated mineral oils carcinogenic to humans, although the evidence for carcinogenicity of highly-refined oils is inadequate. Long-term animal studies of USP grade mineral oil would be pertinent, to determine if this irritant compound would be carcinogenic despite the fact that it contains no measureable PAH. As has been pointed out, while the presence of PAH is strong evidence for carcinogenic potential, the absence of PAH does not preclude tumorigenic activity (Bingham *et al.,*1980).

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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 Black Newsprint Inks[1]

	CU	USPMO	PIMO	LIMIX	OIMIX
MALE					
n	10	10	10	10	10
Necropsy body wt	341 ± 6	336 ± 4	332 ± 6	332 ± 3	327 ± 5
Heart					
Absolute	0.98 ± 0.02	1.00 ± 0.02	1.01 ± 0.02	1.00 ± 0.02	1.00 ± 0.03
Relative	2.87 ± 0.05	2.99 ± 0.05	3.05 ± 0.04	3.00 ± 0.08	3.05 ± 0.05
Right Kidney					
Absolute	1.23 ± 0.03	1.34 ± 0.02*	1.23 ± 0.03	1.21 ± 0.02	1.22 ± 0.04
Relative	3.62 ± 0.07	3.98 ± 0.07**	3.71 ± 0.05	3.66 ± 0.06	3.73 ± 0.08
Liver					
Absolute	13.99 ± 0.44	16.01 ± 0.43**	14.61 ± 0.38	13.49 ± 0.29	13.76 ± 0.52
Relative	40.9 ± 0.9	47.7 ± 1.3**	44.0 ± 0.9	40.7 ± 0.9	42.0 ± 1.3
Lungs					
Absolute	1.95 ± 0.09	1.90 ± 0.06	1.91 ± 0.08	1.85 ± 0.05	1.91 ± 0.07
Relative	5.72 ± 0.29	5.66 ± 0.18	5.78 ± 0.25	5.58 ± 0.18	5.83 ± 0.21
Right Testis					
Absolute	1.41 ± 0.03	1.40 ± 0.02	1.42 ± 0.02	1.42 ± 0.02	1.41 ± 0.03
Relative	4.13 ± 0.05	4.17 ± 0.05	4.28 ± 0.07	4.28 ± 0.06	4.29 ± 0.05
Thymus					
Absolute	0.34 ± 0.02	0.37 ± 0.02	0.32 ± 0.02	0.33 ± 0.02	0.31 ± 0.02
Relative	0.99 ± 0.04	1.09 ± 0.05	0.97 ± 0.06	0.99 ± 0.05	0.93 ± 0.05
FEMALE					
n	10	10	10	10	10
Necropsy body wt	195 ± 2	192 ± 2	183 ± 2**	176 ± 3**	185 ± 2*
Heart					
Absolute	0.67 ± 0.02	0.67 ± 0.01	0.64 ± 0.01	0.62 ± 0.01*	0.65 ± 0.01
Relative	3.43 ± 0.08	3.48 ± 0.05	3.50 ± 0.03	3.52 ± 0.10	3.52 ± 0.09
Right Kidney					
Absolute	0.74 ± 0.01	0.83 ± 0.01**	0.71 ± 0.01	0.70 ± 0.02	0.74 ± 0.01
Relative	3.80 ± 0.07	4.29 ± 0.05**	3.89 ± 0.05	3.96 ± 0.05	3.98 ± 0.06
Liver					
Absolute	7.27 ± 0.17	8.32 ± 0.15**	7.15 ± 0.13	6.86 ± 0.15	7.12 ± 0.12
Relative	37.3 ± 0.7	43.3 ± 0.9**	39.0 ± 0.6	38.9 ± 0.4	38.5 ± 0.7
Lungs					
Absolute	1.42 ± 0.07	1.41 ± 0.05	1.27 ± 0.05	1.19 ± 0.05*	1.35 ± 0.06
Relative	7.27 ± 0.31	7.34 ± 0.26	6.94 ± 0.28	6.75 ± 0.27	7.29 ± 0.32
Thymus					
Absolute	0.27 ± 0.02	0.26 ± 0.01	0.26 ± 0.02	0.21 ± 0.01	0.24 ± 0.02
Relative	1.37 ± 0.11	1.35 ± 0.05	1.41 ± 0.10	1.21 ± 0.07	1.32 ± 0.08

[1] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error); CU=clipped, untreated; USPMO=USP grade mineral oil; PIMO=printing ink mineral oil; LIMIX=letterpress ink mixture; OIMIX=offset ink mixture.

* Statistically significantly different (P≤0.05) from the control group by Dunnett's test.

** Statistically significantly different (P≤0.01) from the control group by 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 Black Newsprint Inks: I. Mineral Oil[1]

	CU	USPMO	PIMO
MALE			
n	10	10	10
Necropsy body wt	32.7 ± 1.1	33.5 ± 0.7	34.2 ± 0.8
Heart			
Absolute	0.148 ± 0.006	0.168 ± 0.006	0.174 ± 0.010*
Relative	4.56 ± 0.20	5.03 ± 0.21	5.12 ± 0.36
Right Kidney			
Absolute	0.374 ± 0.012	0.391 ± 0.011	0.401 ± 0.009
Relative	11.5 ± 0.2	11.7 ± 0.3	11.7 ± 0.3
Liver			
Absolute	1.70 ± 0.05	1.87 ± 0.03*	2.00 ± 0.04**
Relative	52.0 ± 1.0	55.8 ± 1.2	58.5 ± 1.3**
Lungs			
Absolute	0.251 ± 0.011	0.271 ± 0.021	0.279 ± 0.013
Relative	7.74 ± 0.39	8.15 ± 0.69	8.18 ± 0.39
Right Testis			
Absolute	0.095 ± 0.002	0.094 ± 0.002	0.097 ± 0.001
Relative	2.91 ± 0.07	2.80 ± 0.06	2.85 ± 0.05
Thymus			
Absolute	0.023 ± 0.002	0.028 ± 0.002	0.030 ± 0.003*
Relative	0.70 ± 0.05	0.84 ± 0.05	0.88 ± 0.07
FEMALE			
n	10	10	10
Necropsy body wt	29.7 ± 1.1	30.4 ± 0.7	31.9 ± 1.0
Heart			
Absolute	0.156 ± 0.010	0.149 ± 0.005	0.154 ± 0.006
Relative	5.28 ± 0.31	4.92 ± 0.17	4.86 ± 0.18
Right Kidney			
Absolute	0.228 ± 0.008	0.249 ± 0.005*	0.243 ± 0.005
Relative	7.76 ± 0.29	8.21 ± 0.17	7.67 ± 0.17
Liver			
Absolute	1.59 ± 0.07	1.76 ± 0.03	1.84 ± 0.05**
Relative	53.6 ± 1.3	58.2 ± 1.2	58.1 ± 1.7
Lungs			
Absolute	0.243 ± 0.015	0.245 ± 0.010	0.253 ± 0.010
Relative	8.20 ± 0.38	8.05 ± 0.24	8.00 ± 0.37
Thymus			
Absolute	0.034 ± 0.003	0.039 ± 0.002	0.036 ± 0.003
Relative	1.13 ± 0.07	1.28 ± 0.07	1.13 ± 0.06

[1] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error); CU=clipped, untreated; USPMO=USP grade mineral oil; PIMO=printing ink mineral oil.

* Statistically significantly different (P≤0.05) from the control group by Dunnett's test.

** Statistically significantly different (P≤0.01) from the control group by Dunnett's test.

TABLE A3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Dermal Study of Black Newsprint Inks: II. Letterpress Ink[1]

	CU	LI-A	LI-B	LI-C	LI-D	LIMIX
MALE						
n	10	10	10	10	10	10
Necropsy body wt	32.7 ± 1.1	34.0 ± 0.6	33.6 ± 0.6	34.1 ± 0.6	34.4 ± 0.9	33.6 ± 0.8
Heart						
Absolute	0.148 ± 0.006	0.180 ± 0.008**	0.173 ± 0.008*	0.173 ± 0.004*	0.166 ± 0.009	0.156 ± 0.004
Relative	4.56 ± 0.20	5.34 ± 0.32	5.13 ± 0.18	5.11 ± 0.15	4.87 ± 0.36	4.66 ± 0.08
Right Kidney						
Absolute	0.374 ± 0.012	0.409 ± 0.012	0.385 ± 0.014	0.399 ± 0.010	0.397 ± 0.010	0.402 ± 0.014
Relative	11.5 ± 0.2	12.0 ± 0.2	11.5 ± 0.3	11.7 ± 0.4	11.6 ± 0.3	12.0 ± 0.2
Liver						
Absolute	1.70 ± 0.05	1.94 ± 0.06*	1.89 ± 0.05	2.00 ± 0.05**	1.85 ± 0.07	2.02 ± 0.07**
Relative	52.0 ± 1.0	57.2 ± 1.4*	56.4 ± 1.5	58.6 ± 1.2**	53.8 ± 1.9	60.1 ± 1.3**
Lungs						
Absolute	0.251 ± 0.011	0.273 ± 0.012	0.265 ± 0.013	0.241 ± 0.10	0.285 ± 0.010	0.268 ± 0.012
Relative	7.74 ± 0.39	8.09 ± 0.44	7.90 ± 0.34	7.12 ± 0.39	8.38 ± 0.45	7.98 ± 0.28
Right Testis						
Absolute	0.094 ± 0.002	0.101 ± 0.002	0.096 ± 0.002	0.096 ± 0.003	0.101 ± 0.004	0.098 ± 0.002
Relative	2.91 ± 0.07	2.98 ± 0.04	2.87 ± 0.06	2.82 ± 0.10	2.93 ± 0.09	2.92 ± 0.09
Thymus						
Absolute	0.023 ± 0.002	0.029 ± 0.003	0.027 ± 0.002	0.029 ± 0.003	0.030 ± 0.003	0.027 ± 0.002
Relative	0.70 ± 0.05	0.86 ± 0.08	0.81 ± 0.04	0.85 ± 0.07	0.88 ± 0.09	0.79 ± 0.06
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	29.7 ± 1.1	30.2 ± 1.1	29.6 ± 0.5	30.2 ± 0.9	30.6 ± 1.0	29.9 ± 0.6
Heart						
Absolute	0.156 ± 0.010	0.155 ± 0.005	0.148 ± 0.008	0.154 ± 0.009	0.171 ± 0.010	0.155 ± 0.005
Relative	5.28 ± 0.31	5.18 ± 0.25	5.02 ± 0.26	5.13 ± 0.37	5.62 ± 0.36	5.20 ± 0.17
Right Kidney						
Absolute	0.228 ± 0.008	0.237 ± 0.012	0.243 ± 0.004	0.246 ± 0.006	0.242 ± 0.007	0.233 ± 0.017
Relative	7.76 ± 0.29	7.84 ± 0.28	8.26 ± 0.18	8.19 ± 0.24	7.94 ± 0.22	7.80 ± 0.57
Liver						
Absolute	1.59 ± 0.06	1.77 ± 0.07	1.76 ± 0.04	1.99 ± 0.04**	1.79 ± 0.06*	1.83 ± 0.04**
Relative	53.6 ± 1.3	58.8 ± 1.3	59.7 ± 1.2*	66.1 ± 1.3**	58.7 ± 1.7	61.5 ± 1.8**
Lungs						
Absolute	0.243 ± 0.015	0.238 ± 0.007	0.251 ± 0.008	0.270 ± 0.013	0.254 ± 0.012	0.246 ± 0.009
Relative	8.20 ± 0.38	7.95 ± 0.28	8.53 ± 0.30	8.97 ± 0.45	8.34 ± 0.38	8.21 ± 0.26
Thymus						
Absolute	0.034 ± 0.003	0.040 ± 0.003	0.039 ± 0.003	0.036 ± 0.003	0.036 ± 0.002	0.037 ± 0.002
Relative	1.13 ± 0.07	1.31 ± 0.08	1.30 ± 0.10	1.17 ± 0.09	1.16 ± 0.06	1.26 ± 0.10

[1] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error); CU=clipped, untreated; LI-A=letterpress ink, lot A; LI-B=letterpress ink, lot B; LI-C=letterpress ink, lot C; LI-D=letterpress ink, lot D; LIMIX=letterpress ink mixture.

* Statistically significantly different (P≤0.05) from the control group by Dunnett's test.

** Statistically significantly different (P≤0.01) from the control group by Dunnett's test.

TABLE A4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Dermal Study of Black Newsprint Inks: III. Offset Ink[1]

	CU	OI-E	OI-F	OI-G	OI-H	OIMIX
MALE						
n	10	10	10	10	10	10
Necropsy body wt	32.7 ± 1.1	33.6 ± 0.8	32.8 ± 0.7	32.6 ± 0.7	32.9 ± 0.7	32.9 ± 1.1
Heart						
Absolute	0.148 ± 0.006	0.165 ± 0.007	0.177 ± 0.009	0.164 ± 0.011	0.165 ± 0.007	0.175 ± 0.010
Relative	4.56 ± 0.20	4.93 ± 0.20	5.40 ± 0.28	5.07 ± 0.35	5.01 ± 0.20	5.40 ± 0.39
Right Kidney						
Absolute	0.374 ± 0.012	0.399 ± 0.013	0.390 ± 0.012	0.373 ± 0.008	0.395 ± 0.009	0.376 ± 0.016
Relative	11.5 ± 0.2	11.9 ± 0.3	11.9 ± 0.2	11.5 ± 0.2	12.0 ± 0.2	11.4 ± 0.2
Liver						
Absolute	1.70 ± 0.05	1.91 ± 0.04*	1.86 ± 0.06	1.83 ± 0.06	1.91 ± 0.05*	1.93 ± 0.06*
Relative	52.0 ± 1.0	56.8 ± 0.6*	56.7 ± 1.2*	56.1 ± 1.4*	57.9 ± 1.1**	58.9 ± 1.0**
Lungs						
Absolute	0.251 ± 0.011	0.277 ± 0.014	0.288 ± 0.024	0.273 ± 0.014	0.293 ± 0.012	0.275 ± 0.013
Relative	7.74 ± 0.39	8.24 ± 0.36	8.75 ± 0.66	8.39 ± 0.37	8.95 ± 0.42	8.42 ± 0.41
Right Testis						
Absolute	0.095 ± 0.002	0.099 ± 0.003	0.099 ± 0.002	0.093 ± 0.002	0.100 ± 0.003	0.089 ± 0.008
Relative	2.91 ± 0.07	2.94 ± 0.07	3.01 ± 0.06	2.86 ± 0.06	3.03 ± 0.08	2.71 ± 0.22
Thymus						
Absolute	0.023 ± 0.002	0.031 ± 0.002	0.032 ± 0.003*	0.027 ± 0.003	0.027 ± 0.002	0.024 ± 0.002
Relative	0.70 ± 0.05	0.92 ± 0.08	0.98 ± 0.08*	0.83 ± 0.09	0.82 ± 0.06	0.72 ± 0.04
FEMALE						
n	10	9	10	10	10	10
Necropsy body wt	29.7 ± 1.1	30.0 ± 1.0	30.1 ± 0.7	29.9 ± 0.8	29.9 ± 0.4	29.6 ± 0.6
Heart						
Absolute	0.156 ± 0.010	0.161 ± 0.009	0.154 ± 0.008	0.166 ± 0.011	0.150 ± 0.005	0.148 ± 0.005
Relative	5.28 ± 0.31	5.35 ± 0.18	5.18 ± 0.33	5.61 ± 0.43	5.02 ± 0.15	5.04 ± 0.23
Right Kidney						
Absolute	0.228 ± 0.008	0.236 ± 0.013	0.253 ± 0.006	0.255 ± 0.006	0.253 ± 0.006	0.229 ± 0.010
Relative	7.76 ± 0.29	7.88 ± 0.41	8.45 ± 0.24	8.54 ± 0.16	8.47 ± 0.22	7.73 ± 0.28
Liver						
Absolute	1.59 ± 0.07	1.84 ± 0.10*	1.76 ± 0.05	1.88 ± 0.07*	1.73 ± 0.04	1.78 ± 0.06
Relative	53.6 ± 1.3	61.1 ± 2.8*	58.7 ± 1.3	62.9 ± 1.8**	57.9 ± 1.7	60.2 ± 1.8*
Lungs						
Absolute	0.243 ± 0.015	0.249 ± 0.014	0.247 ± 0.078	0.275 ± 0.016	0.252 ± 0.09	0.255 ± 0.011
Relative	8.20 ± 0.38	8.27 ± 0.34	8.27 ± 0.35	9.19 ± 0.44	8.42 ± 0.30	8.66 ± 0.40
Thymus						
Absolute	0.034 ± 0.003	0.034 ± 0.001	0.036 ± 0.003	0.034 ± 0.004	0.037 ± 0.002	0.034 ± 0.002
Relative	1.13 ± 0.07	1.14 ± 0.06	1.21 ± 0.08	1.14 ± 0.11	1.25 ± 0.08	1.17 ± 0.08

[1] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error); CU=clipped, untreated; OI-E=offset ink, lot E; OI-F=offset ink, lot F; OI-G=offset ink, lot G; OI-H=offset ink, lot H; OIMIX=offset ink mixture.

* Statistically significantly different (P≤0.05) from the control group by Dunnett's test.

** Statistically significantly different (P≤0.01) from the control group by Dunnett's test.

APPENDIX B

Hematology and Clinical Chemistry

TABLE B1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Dermal Study of Black Newsprint Inks[1]

Analysis	CU	USPMO	PIMO	LIMIX	OIMIX
MALE					
n	10	9	9	10	10
Hematology					
Hematocrit (%)	50.5 ± 0.6	50.5 ± 0.7	50.0 ± 0.6	50.0 ± 0.9	49.8 ± 0.9
Hemoglobin (g/dL)	15.9 ± 0.3	16.2 ± 0.3	15.8 ± 0.3	15.8 ± 0.3	16.0 ± 0.3[2]
Erythrocytes (10^6/μL)	9.19 ± 0.08	9.08 ± 0.13	9.01 ± 0.12	9.23 ± 0.11	9.15 ± 0.18
Reticulocytes (10^6/μL)	0.16 ± 0.01	0.19 ± 0.01	0.14 ± 0.01	0.17 ± 0.01	0.13 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.04 ± 0.02	0.00 ± 0.00	0.02 ± 0.01	0.03 ± 0.02	0.01 ± 0.01
Mean cell volume (fL)	54.8 ± 0.5	55.6 ± 0.5	55.3 ± 0.7	54.1 ± 0.5	54.4 ± 0.5
Mean cell hemoglobin (pg)	17.3 ± 0.3	17.9 ± 0.3	17.6 ± 0.3	17.1 ± 0.2	17.5 ± 0.2[2]
Mean cell hemoglobin concentration (g/dL)	31.7 ± 0.3	32.1 ± 0.3	31.8 ± 0.3	31.5 ± 0.2	32.0 ± 0.3[2]
Platelets (10^3/μL)	582.2 ± 13.4	585.8 ± 8.7	580.4 ± 10.9	595.5 ± 13.5	546.2 ± 17.6[2]
Leukocytes (10^3/μL)	7.12 ± 0.35	8.37 ± 0.49	6.90 ± 0.41	7.15 ± 0.35	6.31 ± 0.26
Segmented neutrophils (10^3/μL)	1.23 ± 0.08	1.26 ± 0.15	1.39 ± 0.14	1.21 ± 0.09	1.34 ± 0.15
Lymphocytes (10^3/μL)	5.76 ± 0.33	6.92 ± 0.48	5.41 ± 0.34	5.87 ± 0.29	4.85 ± 0.19
Monocytes (10^3/μL)	0.04 ± 0.02	0.08 ± 0.04	0.03 ± 0.02	0.00 ± 0.00	0.02 ± 0.01
Eosinophils (10^3/μL)	0.09 ± 0.02	0.11 ± 0.04	0.08 ± 0.02	0.06 ± 0.02	0.11 ± 0.03
Clinical Chemistry					
Methemoglobin (%)	1.99 ± 0.05	1.97 ± 0.04	1.98 ± 0.07	2.25 ± 0.22	2.12 ± 0.08[2]
FEMALE					
n	10	10	10	10	9
Hematology					
Hematocrit (%)	48.1 ± 0.5	48.3 ± 0.5	48.2 ± 0.4	47.8 ± 0.5	48.2 ± 0.6
Hemoglobin (g/dL)	14.7 ± 0.2	14.9 ± 0.2	14.7 ± 0.1	14.5 ± 0.2	14.7 ± 0.2
Erythrocytes (10^6/μL)	8.26 ± 0.12	8.28 ± 0.09	8.25 ± 0.09	8.13 ± 0.08	8.27 ± 0.12
Reticulocytes (10^6/μL)	0.14 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.14 ± 0.02
Nucleated erythrocytes (10^3/μL)	0.04 ± 0.02	0.07 ± 0.02	0.08 ± 0.02	0.07 ± 0.02	0.05 ± 0.02
Mean cell volume (fL)	58.2 ± 0.3	58.3 ± 0.2	58.4 ± 0.3	58.9 ± 0.3	58.4 ± 0.3
Mean cell hemoglobin (pg)	17.9 ± 0.1	18.0 ± 0.1	17.9 ± 0.1	17.9 ± 0.1	17.8 ± 0.1
Mean cell hemoglobin concentration (g/dL)	30.7 ± 0.2	30.9 ± 0.2	30.6 ± 0.2	30.4 ± 0.3	30.5 ± 0.2
Platelets (10^3/μL)	602.7 ± 18.7	569.8 ± 21.4	596.5 ± 13.6	572.9 ± 13.8	609.4 ± 10.5
Leukocytes (10^3/μL)	8.08 ± 0.35	8.05 ± 0.29	7.61 ± 0.37	6.97 ± 0.33	7.30 ± 0.55
Segmented neutrophils (10^3/μL)	1.58 ± 0.12	1.53 ± 0.13	1.26 ± 0.10	1.39 ± 0.14	1.43 ± 0.19
Lymphocytes (10^3/μL)	6.28 ± 0.37	6.26 ± 0.32	6.20 ± 0.33	5.38 ± 0.28	5.69 ± 0.39
Monocytes (10^3/μL)	0.10 ± 0.03	0.14 ± 0.03	0.07 ± 0.03	0.12 ± 0.03	0.08 ± 0.02
Eosinophils (10^3/μL)	0.12 ± 0.03	0.13 ± 0.02	0.08 ± 0.03	0.09 ± 0.02	0.10 ± 0.03
Clinical Chemistry					
Methemoglobin (%)	1.86 ± 0.05	1.77 ± 0.08	1.99 ± 0.05	1.90 ± 0.05	1.79 ± 0.06

[1] Mean ± standard error. CU=clipped, untreated; USPMO=USP grade mineral oil; PIMO=printing ink mineral oil; LIMIX=letterpress ink mixture; OIMIX=offset ink mixture. Differences from the control group are not significant by Dunn's test.

[2] n=9.

TABLE B2 Hematology and Clinical Chemistry Data for B6C3F$_1$ Mice in the 13-Week Dermal Study of Black Newsprint Inks: I. Mineral Oil[1]

Analysis	CU	USPMO	PIMO
MALE			
n	10	10	10
Hematology			
Hematocrit (%)	50.7 ± 0.8	50.7 ± 0.8	49.9 ± 0.3
Hemoglobin (g/dL)	15.5 ± 0.2	15.1 ± 0.2	15.2 ± 0.2
Erythrocytes (10^6/μL)	9.4 ± 0.1	9.3 ± 0.1	9.1 ± 0.1
Reticulocytes (10^6/μL)	0.17 ± 0.03	0.15 ± 0.02	0.19 ± 0.02
Mean cell volume (fL)	54.2 ± 0.5	54.7 ± 0.5	55.0 ± 0.2
Mean cell hemoglobin (pg)	16.6 ± 0.2	16.3 ± 0.1	16.8 ± 0.1
Mean cell hemoglobin concentration (g/dL)	30.6 ± 0.3	29.8 ± 0.3	30.6 ± 0.2
Platelets (10^3/μL)	1042.9 ± 46.3	1005.1 ± 23.5	1005.4 ± 27.9
Leukocytes (10^3/μL)	6.34 ± 0.34	6.87 ± 0.70	5.16 ± 0.40
Segmented neutrophils (10^3/μL)	2.19 ± 0.25	2.87 ± 0.37	1.66 ± 0.15
Lymphocytes (10^3/μL)	4.11 ± 0.23	3.98 ± 0.45	3.46 ± 0.30
Eosinophils (10^3/μL)	0.04 ± 0.02	0.03 ± 0.01	0.04 ± 0.01
Clinical Chemistry			
Methemoglobin (%)	1.45 ± 0.13	1.48 ± 0.11	1.53 ± 0.05
FEMALE			
n	10	10	10
Hematology			
Hematocrit (%)	51.4 ± 1.2	49.2 ± 0.5	50.3 ± 0.5
Hemoglobin (g/dL)	15.4 ± 0.4	14.7 ± 0.2	15.3 ± 0.2
Erythrocytes (10^6/μL)	9.4 ± 0.2	8.9 ± 0.1	9.2 ± 0.1
Reticulocytes (10^6/μL)	0.20 ± 0.02	0.15 ± 0.01	0.18 ± 0.02
Mean cell volume (fL)	54.8 ± 0.4	55.0 ± 0.3	54.5 ± 0.4
Mean cell hemoglobin (pg)	16.4 ± 0.1	16.5 ± 0.1	16.6 ± 0.2
Mean cell hemoglobin concentration (g/dL)	30.0 ± 0.2	30.0 ± 0.2	30.4 ± 0.2
Platelets (10^3/μL)	985.1 ± 42.6	1049.4 ± 22.6	1008.5 ± 33.5
Leukocytes (10^3/μL)	7.34 ± 0.41	7.36 ± 0.28	6.63 ± 0.25
Segmented neutrophils (10^3/μL)	2.44 ± 0.27	2.51 ± 0.20	2.31 ± 0.22
Lymphocytes (10^3/μL)	4.79 ± 0.30	4.77 ± 0.28	4.26 ± 0.15
Monocytes (10^3/μL)	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
Eosinophils (10^3/μL)	0.10 ± 0.03	0.07 ± 0.02	0.05 ± 0.02
Clinical Chemistry			
Methemoglobin (%)	1.47 ± 0.08	1.43 ± 0.10	1.32 ± 0.09

[1] Mean ± standard error. CU=clipped, untreated; USPMO=USP grade mineral oil; PIMO=printing ink mineral oil. Differences from the control group are not significant by Dunn's test.

TABLE B3 Hematology and Clinical Chemistry Data for B6C3F$_1$ Mice in the 13-Week Dermal Study of Black Newsprint Inks: II. Letterpress Ink[1]

Analysis	CU	LI-A	LI-B	LI-C	LI-D	LIMIX
MALE						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)	50.7 ± 0.8	50.9 ± 0.7	49.9 ± 0.9	49.6 ± 0.4	49.1 ± 0.4	49.9 ± 0.4
Hemoglobin (g/dL)	15.5 ± 0.2	15.5 ± 0.2	15.2 ± 0.3	15.0 ± 0.1	15.1 ± 0.2	15.2 ± 0.2
Erythrocytes (10^6/μL)	9.36 ± 0.14	9.31 ± 0.11	9.23 ± 0.10	8.96 ± 0.11	9.07 ± 0.08	9.20 ± 0.08
Reticulocytes (10^6/μL)	0.17 ± 0.03	0.17 ± 0.02	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.15 ± 0.01
Mean cell volume (fL)	54.2 ± 0.5	54.6 ± 0.3	54.0 ± 0.5	55.3 ± 0.6	54.0 ± 0.3	54.3 ± 0.3
Mean cell hemoglobin (pg)	16.6 ± 0.2	16.7 ± 0.1	16.5 ± 0.2	16.7 ± 0.2	16.7 ± 0.1	16.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)	30.6 ± 0.3	30.5 ± 0.3	30.5 ± 0.3	30.2 ± 0.2	30.8 ± 0.2	30.4 ± 0.2
Platelets (10^3/μL)	1042.9 ± 46.3	1010.2 ± 18.2	993.3 ± 37.4	976.4 ± 27.7	932.4 ± 26.5	1001.4 ± 19.9
Leukocytes (10^3/μL)	6.34 ± 0.34	7.70 ± 0.97	6.13 ± 0.63	6.36 ± 0.63	5.87 ± 0.39	6.70 ± 0.59
Segmented neutrophils (10^3/μL)	2.19 ± 0.25	3.40 ± 0.72	2.47 ± 0.44	2.41 ± 0.38	2.22 ± 0.23	2.27 ± 0.25
Lymphocytes (10^3/μL)	4.11 ± 0.23	4.27 ± 0.32	3.59 ± 0.24	3.89 ± 0.29	3.63 ± 0.30	4.40 ± 0.44
Eosinophils (10^3/μL)	0.04 ± 0.02	0.03 ± 0.01	0.07 ± 0.01	0.06 ± 0.02	0.02 ± 0.01	0.03 ± 0.01
Clinical Chemistry						
Methemoglobin (%)	1.45 ± 0.13	2.73 ± 1.27	1.60 ± 0.11	1.43 ± 0.08	1.53 ± 0.06	1.50 ± 0.05
FEMALE						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)	51.4 ± 1.2	48.9 ± 0.3	49.7 ± 0.4	48.7 ± 0.4*	49.2 ± 0.4	49.2 ± 0.6
Hemoglobin (g/dL)	15.4 ± 0.4	14.9 ± 0.1	15.0 ± 0.1	14.9 ± 0.1	15.1 ± 0.2	14.9 ± 0.2
Erythrocytes (10^6/μL)	9.37 ± 0.23	8.99 ± 0.10	9.04 ± 0.05	8.86 ± 0.09	8.96 ± 0.10	8.99 ± 0.13
Reticulocytes (10^6/μL)	0.20 ± 0.02	0.16 ± 0.01	0.15 ± 0.02	0.15 ± 0.01	0.15 ± 0.01	0.17 ± 0.02
Mean cell volume (fL)	54.8 ± 0.4	54.5 ± 0.6	55.0 ± 0.3	55.1 ± 0.5	55.0 ± 0.3	54.8 ± 0.5
Mean cell hemoglobin (pg)	16.4 ± 0.1	16.5 ± 0.2	16.6 ± 0.1	16.8 ± 0.1*	16.8 ± 0.1*	16.6 ± 0.1

TABLE B3 Hematology and Clinical Chemistry Data for B6C3F$_1$ Mice in the 13-Week Dermal Study of Black Newsprint Inks: II. Letterpress Ink (continued)

Analysis	CU	LI-A	LI-B	LI-C	LI-D	LIMIX
FEMALE (continued)						
Hematology (continued)						
Mean cell hemoglobin concentration (g/dL)	30.0 ± 0.2	30.4 ± 0.2	30.2 ± 0.2	30.6 ± 0.2	30.6 ± 0.2*	30.3 ± 0.2
Platelets (10^3/μL)	985.1 ± 42.6	966.8 ± 24.2	990.4 ± 31.7	1018.8 ± 31.9	963.4 ± 19.7	962.4 ± 22.8
Leukocytes (10^3/μL)	7.34 ± 0.41	7.07 ± 0.38	7.65 ± 0.35	6.84 ± 0.20	6.57 ± 0.17	5.88 ± 0.31*
Segmented neutrophils (10^3/μL)	2.44 ± 0.27	2.63 ± 0.18	3.28 ± 0.36	2.67 ± 0.14	2.24 ± 0.14	2.12 ± 0.19
Lymphocytes (10^3/μL)	4.79 ± 0.30	4.39 ± 0.27	4.31 ± 0.18	4.11 ± 0.15	4.25 ± 0.13	3.71 ± 0.22**
Monocytes (10^3/μL)	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.00 ± 0.00
Eosinophils (10^3/μL)	0.10 ± 0.03	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.02	0.05 ± 0.02	0.05 ± 0.01
Clinical Chemistry						
Methemoglobin (%)	1.47 ± 0.08	1.38 ± 0.08	1.30 ± 0.07	1.36 ± 0.11	1.35 ± 0.11	1.36 ± 0.09

[1] Mean ± standard error. CU=clipped, untreated; LI-A=letterpress ink, lot A; LI-B=letterpress ink, lot B; LI-C=letterpress ink, lot C; LI-D=letterpress ink, lot D; LIMIX=letterpress ink mixture.

* Statistically significantly different (P≤0.05) from the control group by Dunn's test.

** Statistically significantly different (P≤0.01) from the control group by Dunn's test.

TABLE B4 Hematology and Clinical Chemistry Data for B6C3F$_1$ Mice in the 13-Week Dermal Study of Black Newsprint Inks: III. Offset Ink[1]

Analysis	CU	OI-E	OI-F	OI-G	OI-H	OIMIX
MALE						
n	10	10	10	10	10	10
Hematology						
Hematocrit (%)	50.7 ± 0.8	51.1 ± 0.7	50.4 ± 1.0	49.0 ± 0.7	50.5 ± 0.8	49.6 ± 0.5
Hemoglobin (g/dL)	15.5 ± 0.2	15.5 ± 0.2	15.4 ± 0.2	15.1 ± 0.2	15.3 ± 0.2	15.2 ± 0.2
Erythrocytes (10^6/μL)	9.36 ± 0.14	9.31 ± 0.09	9.24 ± 0.16	9.04 ± 0.12	9.11 ± 0.12	9.13 ± 0.09
Reticulocytes (10^6/μL)	0.17 ± 0.03	0.17 ± 0.01	0.15 ± 0.01	0.16 ± 0.02	0.16 ± 0.01	0.16 ± 0.01
Mean cell volume (fL)	54.2 ± 0.5	54.9 ± 0.5	54.6 ± 0.3	54.3 ± 0.3	55.4 ± 0.3	54.4 ± 0.3
Mean cell hemoglobin (pg)	16.6 ± 0.2	16.7 ± 0.0	16.6 ± 0.1	16.7 ± 0.1	16.8 ± 0.1	16.6 ± 0.2
Mean cell hemoglobin concentration (g/dL)	30.6 ± 0.3	30.4 ± 0.2	30.5 ± 0.3	30.7 ± 0.3	30.3 ± 0.3	30.6 ± 0.3
Platelets (10^3/μL)	1042.9 ± 46.3	983.6 ± 33.9	984.3 ± 17.8	1053.1 ± 41.6	972.2 ± 45.2	969.9 ± 45.1
Leukocytes (10^3/μL)	6.34 ± 0.34	6.66 ± 0.49	6.38 ± 0.33	5.54 ± 0.58	6.32 ± 0.59	6.56 ± 0.37
Segmented neutrophils (10^3/μL)	2.19 ± 0.25	2.59 ± 0.22	2.28 ± 0.17	2.02 ± 0.30	2.44 ± 0.29	2.49 ± 0.20
Lymphocytes (10^3/μL)	4.11 ± 0.23	3.99 ± 0.36	4.02 ± 0.29	3.48 ± 0.34	3.86 ± 0.34	4.04 ± 0.27
Eosinophils (10^3/μL)	0.04 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.02
Clinical Chemistry						
Methemoglobin (%)	1.45 ± 0.13	1.60 ± 0.06	1.40 ± 0.10	1.39 ± 0.08	1.49 ± 0.09	1.59 ± 0.10
FEMALE						
n	10	9	10	10	10	10
Hematology						
Hematocrit (%)	51.4 ± 1.2	49.8 ± 1.7	50.3 ± 0.7	48.3 ± 0.5	49.3 ± 0.5	50.4 ± 0.6
Hemoglobin (g/dL)	15.4 ± 0.4	15.1 ± 0.5	15.1 ± 0.2	14.6 ± 0.1	15.0 ± 0.1	15.2 ± 0.2
Erythrocytes (10^6/μL)	9.37 ± 0.23	9.12 ± 0.33	9.13 ± 0.13	8.75 ± 0.08*	8.97 ± 0.11	9.20 ± 0.12
Reticulocytes (10^6/μL)	0.20 ± 0.02	0.17 ± 0.02	0.16 ± 0.02	0.14 ± 0.02	0.16 ± 0.01	0.14 ± 0.01
Mean cell volume (fL)	54.8 ± 0.4	54.6 ± 0.5	54.9 ± 0.2	55.2 ± 0.3	55.1 ± 0.2	54.9 ± 0.3
Mean cell hemoglobin (pg)	16.4 ± 0.1	16.6 ± 0.1	16.6 ± 0.1	16.7 ± 0.1	16.8 ± 0.1	16.6 ± 0.1

TABLE B4 Hematology and Clinical Chemistry Data for B6C3F$_1$ Mice in the 13-Week Dermal Study of Black Newsprint Inks: III. Offset Ink (continued)

Analysis	CU	OI-E	OI-F	OI-G	OI-H	OIMIX
FEMALE (continued)						
Hematology (continued)						
Mean cell hemoglobin concentration (g/dL)	30.0 ± 0.2	30.3 ± 0.2	30.1 ± 0.2	30.3 ± 0.2	30.5 ± 0.2	30.2 ± 0.2
Platelets ($10^3/\mu L$)	985.1 ± 42.6	1021.6 ± 21.9	1062.4 ± 50.1	1012.6 ± 25.6	931.1 ± 29.1	995.2 ± 32.7
Leukocytes ($10^3/\mu L$)	7.34 ± 0.41	7.04 ± 0.69	7.10 ± 0.32	6.41 ± 0.32	7.38 ± 0.60	7.29 ± 0.36
Segmented neutrophils ($10^3/\mu L$)	2.44 ± 0.27	2.88 ± 0.56	2.92 ± 0.21	2.61 ± 0.23	2.80 ± 0.34	3.01 ± 0.28
Lymphocytes ($10^3/\mu L$)	4.79 ± 0.30	4.07 ± 0.27	4.13 ± 0.20	3.79 ± 0.21*	4.52 ± 0.38	4.23 ± 0.26
Monocytes ($10^3/\mu L$)	0.01 ± 0.01	0.02 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.02
Eosinophils ($10^3/\mu L$)	0.10 ± 0.03	0.07 ± 0.02	0.05 ± 0.02	0.02 ± 0.02	0.05 ± 0.02	0.04 ± 0.02
Clinical Chemistry						
Methemoglobin (%)	1.47 ± 0.08	1.21 ± 0.11	1.33 ± 0.09	1.32 ± 0.08	1.37 ± 0.07	1.40 ± 0.09

[1] Mean ± standard error. CU=untreated, clipped; OI-E=offset ink, lot E; OI-F=offset ink, lot F; OI-G-offset ink, lot G; OI-H-offset ink, lot H; OIMIX=offset ink mixture.

* Statistically significantly different ($P \leq 0.05$) from the control group by Dunn's test.

** Statistically significantly different ($P \leq 0.01$) from the control group by Dunn's test.

APPENDIX C

Reproductive Tissue Evaluations and Estrous Cycle Characterization

TABLE C1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Dermal Study of Black Newsprint Inks

Study Parameters[1]	CU	PIMO	LIMIX	OIMIX
Weights (g)				
Necropsy body weight	341 ± 6	332 ± 6	332 ± 3	327 ± 5
Left testicle	1.515 ± 0.014	1.502 ± 0.016	1.519 ± 0.022	1.494 ± 0.022
Left epididymis	0.455 ± 0.004	0.448 ± 0.008	0.440 ± 0.008	0.443 ± 0.007
Left epididymal tail	0.155 ± 0.004	0.162 ± 0.004	0.150 ± 0.005	0.157 ± 0.004
Spermatozoal measurements				
Motility (%)	70 ± 2	72 ± 2	72 ± 2	70 ± 2
Concentration (10^6/g)	558 ± 23	580 ± 44	562 ± 25	523 ± 17
Spermatid heads (10^7/g testis)	8.19 ± 0.51	8.80 ± 0.27	9.13 ± 0.30	7.82 ± 0.33
Spermatid heads (10^7/testis)	12.37 ± 0.72	13.22 ± 0.43	13.86 ± 0.50	11.69 ± 0.55
Spermatid count (mean/10^3 mL suspension)	61.85 ± 3.58	66.10 ± 2.16	69.32 ± 2.50	58.45 ± 2.77

[1] Data presented as mean ± standard error; n=10. CU=clipped, untreated; PIMO=printing ink mineral oil; LIMIX=letterpress ink mixture; OIMIX=offset ink mixture. For reproductive tissue weights, spermatid, and spermatozoal data, differences from the control group are not significant by Dunn's test. For necropsy body weights, differences from the control group are not significant by Dunnett's test.

TABLE C2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Dermal Study of Black Newsprint Inks

Study Parameters[1]	CU	PIMO	LIMIX	OIMIX
Necropsy body weight (g)	195 ± 2	183 ± 2**	176 ± 3**	185 ± 2*
Estrous cycle length (days)	4.95 ± 0.05	5.00 ± 0.00	5.10 ± 0.10	5.00 ± 0.00
Estrous stages as % of cycle				
Diestrus	40.8	37.5	41.7	42.5
Proestrus	19.2	20.0	18.3	17.5
Estrus	25.8	22.5	20.0	23.3
Metestrus	12.5	17.5	18.3	15.0
Uncertain diagnosis	1.7	2.5	1.7	1.7

[1] Data presented as mean ± standard error; n=10. CU=clipped, untreated; PIMO=printing ink mineral oil; LIMIX=letterpress ink mixture; OIMIX=offset ink mixture. Estrous cycle lengths are not significant by Dunn'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.

* Statistically significantly different ($P \leq 0.05$) from the control group by Dunnett's test.

** Statistically significantly different ($P \leq 0.01$) from the control group by Dunnett's test.

APPENDIX D

Genetic Toxicology

TABLE D1 Mutagenicity of Black Newsprint Inks in *Salmonella typhimurium*: I. Letterpress Ink[1]

Strain	Dose (μg/plate)	Revertants/plate[2] -S9 Trial 1	+ hamster S9 5%	+ hamster S9 10%
TA100	0	126 ± 8.4	107 ± 9.9	121 ± 9.0
	100	105 ± 6.4		
	333	90 ± 7.5		
	1000	96 ± 10.6	143 ± 5.7	152 ± 6.3
	1666		157 ± 7.0	164 ± 5.8
	3333	87 ± 6.2	162 ± 6.4	190 ± 21.3
	6666		147 ± 17.9[3]	180 ± 6.4[3]
	10,000	108 ± 9.6[3]	153 ± 5.8[3]	159 ± 17.5[3]
Trial summary		Negative	Equivocal	Weakly Positive
Positive control[4]		493 ± 20.9	895 ± 40.9	625 ± 8.1

Strain	Dose (μg/plate)	Revertants/plate + 30% hamster S9 Trial 1	Trial 2	Trial 3	Trial 4	+ 30% rat S9 Trial 1
TA100	0	133 ± 11.3	120 ± 9.9	162 ± 6.7	111 ± 5.5	142 ± 1.0
	100	127 ± 12.1		157 ± 10.6	116 ± 7.8	148 ± 16.8
	333	127 ± 7.8		171 ± 5.5	119 ± 4.5	122 ± 6.9
	1000	125 ± 11.7	169 ± 14.3	195 ± 9.6	151 ± 2.1	108 ± 7.9
	1666		167 ± 10.4			
	3333	193 ± 16.3	179 ± 12.7	245 ± 19.2	195 ± 7.4	139 ± 18.5
	6666		178 ± 19.6[3]			
	10,000	204 ± 29.1[3]	205 ± 16.2[3]	271 ± 9.8[3]	233 ± 11.9[3]	141 ± 9.1[3]
Trial summary		Weakly Positive	Equivocal	Weakly Positive	Positive	Negative
Positive control		381 ± 14.0	457 ± 11.2	694 ± 45.9	551 ± 8.1	273 ± 2.7

Strain	Dose (μg/plate)	Revertants/plate -S9 Trial 1	+ hamster S9 5% Trial 1	10% Trial 1	30% Trial 1	30% Trial 2	30% Trial 3	30% Trial 4
TA98	0	24 ± 0.9	21 ± 5.0	20 ± 2.7	18 ± 0.9	22 ± 4.7	26 ± 3.5	23 ± 4.2
	100	22 ± 2.9			12 ± 3.4		29 ± 0.3	22 ± 2.2
	333	16 ± 1.9			17 ± 2.1		32 ± 2.5	26 ± 4.6
	1000	16 ± 3.0	31 ± 2.2	35 ± 2.7	13 ± 2.6	42 ± 3.3	51 ± 4.4	28 ± 2.0
	1666		31 ± 5.0	35 ± 1.7		42 ± 1.7		
	3333	17 ± 2.8	31 ± 3.6	44 ± 3.8	15 ± 2.5	48 ± 6.4	75 ± 1.8	48 ± 1.5
	6666		21 ± 2.5[3]	53 ± 7.5[3]		69 ± 7.2[3]	109 ± 2.9[3]	58 ± 4.6[3]
	10,000	18 ± 4.8[3]	30 ± 1.3[3]	45 ± 2.6[3]	46 ± 10.4[3]	67 ± 3.4[3]		
Trial summary		Negative	Negative	Positive	Equivocal	Positive	Positive	Positive
Positive control		526 ± 16.4	649 ± 53.7	468 ± 12.0	213 ± 5.3	196 ± 56.7	419 ± 36.2	331 ± 10.6

TABLE D1 Mutagenicity of Black Newsprint Inks in *Salmonella typhimurium*: I. Letterpress Ink (continued)

Strain	Dose (μg/plate)	Revertants/plate + rat S9 5% Trial 1	10% Trial 1	10% Trial 2	10% Trial 3	30% Trial 1	30% Trial 2
TA98	0	18 ± 3.5	22 ± 1.7	29 ± 1.0	25 ± 4.8	23 ± 1.3	16 ± 3.4
	100			31 ± 3.2	28 ± 1.8	16 ± 0.9	
	333			41 ± 3.3	34 ± 2.0	16 ± 4.0	
	1000	34 ± 3.8	47 ± 9.2	69 ± 4.8	47 ± 5.4	20 ± 0.6	28 ± 8.3
	1666	33 ± 9.4	40 ± 2.9				28 ± 2.9
	3333	38 ± 3.9	47 ± 4.2	94 ± 4.2	55 ± 2.9	21 ± 4.6	32 ± 3.2
	6666	32 ± 2.1[3]	60 ± 0.7[3]	76 ± 1.9[3]	55 ± 3.7[3]		41 ± 2.3[3]
	10,000	21 ± 0.6[3]	32 ± 2.6[3]			33 ± 5.7[3]	26 ± 8.3[3]
Trial summary		Weakly Positive	Positive	Positive	Positive	Negative	Weakly Positive
Positive control		500 ± 6.7	212 ± 33.9	353 ± 11.4	192 ± 20.7	76 ± 6.6	81 ± 5.6

[1] Study performed at SRI International. The detailed protocol is presented in Zeiger *et al.* (1988).
[2] Revertants are presented as mean ± the standard error from three plates.
[3] Precipitate on plate.
[4] The positive controls in the absence of metabolic activation were 4-nitro-*o*-phenylenediamine (TA98) and sodium azide (TA100). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE D2 Mutagenicity of Black Newsprint Inks in *Salmonella typhimurium*: II. Offset Ink[1]

Strain	Dose (μg/plate)	Revertants/plate[2] -S9 Trial 1	+ hamster S9 5% Trial 1	+ hamster S9 10% Trial 1	+ hamster S9 30% Trial 1	+ hamster S9 30% Trial 2	+ hamster S9 30% Trial 3	+ 30% rat S9 Trial 1
TA100	0	114 ± 16.6	129 ± 15.2	143 ± 4.0	128 ± 1.5	115 ± 11.0	132 ± 5.5	168 ± 4.5
	100	89 ± 6.3			130 ± 13.4			157 ± 10.7
	333	98 ± 10.4	131 ± 9.5	119 ± 3.0	118 ± 8.4	116 ± 7.6		149 ± 0.9
	1000	101 ± 11.9	136 ± 9.0	127 ± 12.9	115 ± 9.7	113 ± 5.5	157 ± 15.0	126 ± 7.0
	1666						168 ± 3.8	
	3333	110 ± 1.2	152 ± 6.2	137 ± 5.2	152 ± 21.2	120 ± 7.9	191 ± 16.2	151 ± 14.1
	6666		142 ± 10.3	172 ± 5.2		137 ± 3.2	250 ± 6.9	
	10,000	74 ± 4.0	181 ± 17.3	206 ± 10.5	174 ± 12.9	175 ± 15.8	268 ± 4.7	172 ± 5.0
Trial summary		Negative	Equivocal	Equivocal	Equivocal	Equivocal	Positive	Negative
Positive control[3]		493 ± 20.9	895 ± 40.9	625 ± 8.1	381 ± 14.0	457 ± 11.2	694 ± 45.9	273 ± 2.7

Strain	Dose (μg/plate)	Revertants/plate -S9 Trial 1	+30% hamster S9 Trial 1	+30% hamster S9 Trial 2	+30% hamster S9 Trial 3	30% rat S9 Trial 1	30% rat S9 Trial2	30% rat S9 Trial 3
TA98	0	22 ± 2.6	27 ± 0.7	17 ± 2.1	25 ± 2.7	17 ± 3.8	21 ± 1.0	25 ± 0.6
	100	24 ± 6.6	18 ± 3.2	15 ± 1.2		12 ± 4.1		
	333	20 ± 3.2	30 ± 1.7	18 ± 3.5		14 ± 2.6		
	1000	20 ± 1.9	36 ± 7.5	12 ± 1.3	29 ± 2.9	15 ± 1.5	21 ± 0.9	37 ± 5.4
	1666				32 ± 3.6		25 ± 1.8	34 ± 5.2
	3333	15 ± 0.9	55 ± 7.2	32 ± 3.8	48 ± 1.5	25 ± 6.5	29 ± 1.0	49 ± 8.3
	6666				81 ± 2.2		30 ± 1.5	59 ± 9.4
	10,000	19 ± 1.2	83 ± 26.7	72 ± 2.0	106 ± 11.4	38 ± 11.5	45 ± 2.3	67 ± 14.4
Trial summary		Negative	Positive	Positive	Positive	Equivocal	Equivocal	Positive
Positive control		526 ± 16.4	213 ± 5.3	196 ± 56.7	419 ± 36.2	76 ± 6.6	81 ± 5.6	79 ± 1.9

[1] Study performed at SRI International. The detailed protocol is presented in Zeiger *et al.* (1988).

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

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

APPENDIX E

Analyses of Black Newsprint Ink and Mineral Oil Samples for Polycyclic Aromatic Hydrocarbon (PAHs)

TABLE E1 Analyses of Black Newsprint Ink and Mineral Oil Samples for Polycyclic Aromatic Hydrocarbons (PAHs)

	Concentration (ppm)			
	USPMO[a]	PIMO	LP-MIX	OS-MIX
TARGET PAH				
Napthalene	ND[b]	3	3	3
1-Methylnaphthalene	"	6	2	1
2-Methylnaphthalene	"	~8	2	2
2, 6-Dimethylnaphthalene	"	11	4	2
1, 3-Dimethylnaphthalene	"	10	3	1
1, 6-Dimethylnaphthalene	"	14	9	3
2, 3-Dimethylnaphthalene	"	~6	2	0.7
1, 5-Dimethylnaphthalene	"	1	2	0.3
1, 2-Dimethylnaphthalene	"	~5	3	0.8
Acenaphthylene	"	ND[c]	2	2
Acenaphthene	"	"	2	1
Fluorene	"	12	4	3
Phenanthrene	"	21	16	4
Anthracene	"	ND[c]	1	0.2
2-Methylphenanthrene	"	14	13	4
Fluoranthene	"	1	8	8
Pyrene	"	10	42	43
1-Methylfluorene	"	~23	~17	9
Chrysene	"	14	20	2
1,2-Benzanthracene	"	ND[c]	1	ND[e]
Benzo[a]pyrene	"	1	2	1
Perylene	"	1	4	0.4
2-Nitrofluorene	"	ND[c]	ND[d]	ND[e]
3-Nitrofluorenone	"	"	"	"
9-Nitroanthracene	"	"	"	"
3-Nitropyrene	"	"	"	"
NON-TARGET PAH's				
Methylphenanthrene Isomers	"	39	37	12
Unidentified 5-ring PAH	"	8	~7	2

a Key to samples:
USPMO = USP grade mineral oil;
PIMO = printing ink mineral oil;
LP-MIX = composite mixture of 4 letterpress inks;
OS-MIX = composite mixture of 4 offset inks.

b Not detected; detection limits ranged from 12 to 1284 ppb depending on the analyte.

c Not detected; detection limits: acenaphthylene- 42 ppb; anthracene- 2 ppm; 1, 2-benzanthracene- 625 ppb; 2-nitrofluorene-nitropyrene- 85 ppb.

d Not detected; detection limits: 2-nitrofluorene- 74 ppb; 3-nitrofluorenone- 1208 ppb; 9-nitroanthracene- 106 ppb; 3-nitropyrene-85 ppb.

e Not detected; detection limits: 1, 2-benzanthracene- 250 ppb; 2-nitrofluorene- 65 ppb; 3-nitrofluorenone- 939 ppb; 9-nitroanthracene- 9 ppb; 3-nitropyrene- 23 ppb.