



NTP

National Toxicology Program

U.S. Department of Health and Human Services

NTP TECHNICAL REPORT ON
THE TOXICOLOGY AND
CARCINOGENESIS STUDY OF
TRICLOSAN
(CASRN 3380-34-5)
ADMINISTERED DERMALLY TO
B6C3F1/N MICE

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**NTP Technical Report on the
Toxicology and Carcinogenesis Study of
Triclosan (CASRN 3380-34-5) Administered
Dermally to B6C3F1/N Mice**

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Foreword

The National Toxicology Program (NTP), established in 1978, is an interagency program within the Public Health Service of the U.S. Department of Health and Human Services. Its activities are executed through a partnership of the National Institute for Occupational Safety and Health (part of the Centers for Disease Control and Prevention), the Food and Drug Administration (primarily at the National Center for Toxicological Research), and the National Institute of Environmental Health Sciences (part of the National Institutes of Health), where the program is administratively located. NTP offers a unique venue for the testing, research, and analysis of agents of concern to identify toxic and biological effects, provide information that strengthens the science base, and inform decisions by health regulatory and research agencies to safeguard public health. NTP also works to develop and apply new and improved methods and approaches that advance toxicology and better assess health effects from environmental exposures.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to NTP. The studies described in the NTP Technical Report series are designed and conducted to characterize and evaluate the toxicological potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances (e.g., chemicals, physical agents, and mixtures) selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of commercial production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are derived solely from the results of these NTP studies, and extrapolation of the results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection for study per se is not an indicator of a substance's carcinogenic potential.

NTP conducts its studies in compliance with its laboratory health and safety guidelines and the Food and Drug Administration [Good Laboratory Practice Regulations](#) and meets or exceeds all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the [Public Health Service Policy on Humane Care and Use of Laboratory Animals](#). Studies are subjected to retrospective quality assurance audits before they are presented for public review. Draft reports undergo external peer review before they are finalized and published.

NTP Technical Reports are available free of charge on the [NTP website](#) and cataloged in [PubMed](#), a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health). Data for these studies are included in NTP's [Chemical Effects in Biological Systems](#) database.

For questions about the reports and studies, please email [NTP](#) or call 984-287-3211.

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Explanation of Levels of Evidence of Carcinogenic Activity

The National Toxicology Program (NTP) describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, in as much as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of evidence observed in each experiment: two categories for positive results (**clear evidence and some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been

adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as “were also related” to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as “may have been” related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

Peer Review

The National Toxicology Program (NTP) conducted a peer review of the draft *NTP Technical Report on the Toxicology and Carcinogenesis Study of Triclosan (CASRN 3380-34-5) Administered Dermally to B6C3F1/N Mice* by letter in November 2022 by the experts listed below. Reviewer selection and document review followed established NTP practices. The reviewers were charged to:

1. Peer review the draft NTP Technical Report on the Toxicology and Carcinogenesis Study of Triclosan (CASRN 3380-34-5) Administered Dermally to B6C3F1/N Mice.
2. Comment on NTP's conclusions and interpretations of the data.

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Abstract

Two-year Study in Mice

Triclosan is a broad-spectrum antimicrobial agent to which humans are widely exposed. Very limited data are available regarding the dermal toxicity and the carcinogenic potential of triclosan. In this study, groups of 48 male and 48 female B6C3F1/N mice were untreated or were dermally administered 0 (vehicle), 1.25, 2.7, 5.8, or 12.5 mg triclosan/kg body weight/day (mg/kg/day) in 95% ethanol, 7 days per week for 2 years. Vehicle control animals received 95% ethanol only; untreated, naive control mice were not dosed. There were no significant differences in survival among the groups. The highest dose of triclosan decreased the body weights of mice in both sexes, but the decrease was $\leq 8\%$.

Minimal to mild epidermal hyperplasia (males and females), suppurative inflammation (males only), and ulceration (males only) were observed at the site of application in the dosed groups, with the highest incidence occurring in the 12.5 mg/kg/day groups. In male and female mice, no skin neoplasms were identified at the site of application. In male mice, the two highest dosed groups (5.8 and 12.5 mg/kg/day) had significantly increased incidences of hepatocellular carcinoma, and the incidences occurred with a positive trend relative to the vehicle control group. A positive trend in the incidence of hepatocellular adenoma or carcinoma (combined) was observed in male mice, with significantly increased incidences in the ≥ 2.7 mg/kg/day group. Female mice had a positive trend in the incidence of pancreatic islet adenoma.

Conclusions

Under the conditions of this 2-year dermal study, there was *some evidence of carcinogenic activity* of triclosan in male B6C3F1/N mice based on the increased incidences of hepatocellular adenoma or carcinoma (combined). There was *equivocal evidence of carcinogenic activity* of triclosan in female B6C3F1/N mice based on higher occurrences of pancreatic islet adenomas.

Dermal administration of triclosan resulted in increased incidences of nonneoplastic epidermal lesions at the site of application in male and female mice.

Synonyms: 2,4,4-trichloro-2-hydroxydiphenyl ether, 2,4,4'-trichloro-2'-hydroxydiphenyl ether, trichloro-2'-hydroxydiphenyl ether

Trade names: Irgasan, CH 3565, Irgasan CH 3565, Irgasan DP300, Ster-Zac, Tinosan AM110 Antimicrobial, Invasan DP 300R, Invasan DP 300 TEX, Irgaguard[®] B 1000, VIV-20, Irgacare MP, Lexol 300, Cloxifenolum, Aquasept, Gamophen, Vinyzene DP 7000, Vinyzene SB-30, Sanitized Brand, Microbanish R, Vikol THP, Ultra-Fresh, Microban Additive "B," AerisGuard, and Sapoderm

Summary of the Two-year Carcinogenesis Study of Triclosan

	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Dermal Dose in Ethanol	[Untreated control group], 0, 1.25, 2.7, 5.8, or 12.5 mg/kg/day	[Untreated control group], 0, 1.25, 2.7, 5.8, or 12.5 mg/kg/day
Survival Rates	[31/48], 35/48, 30/48, 34/48, 28/48, 31/48	[33/48], 35/48, 29/48, 31/48, 37/48, 35/48
Body Weights	Dosed groups within 10% of the vehicle control group	Dosed groups within 10% of the vehicle control group
Nonneoplastic Effects	<u>Skin, site of application:</u> epidermis, hyperplasia ([2/48], 1/48, 3/48, 20/48, 17/48, 43/48); epidermis, inflammation, suppurative ([2/48], 0/48, 3/48, 2/48, 4/48, 9/48); epidermis, ulcer ([2/48], 0/48, 2/48, 1/48, 2/48, 5/48)	<u>Skin, site of application:</u> epidermis, hyperplasia ([1/48], 0/48, 5/48, 7/48, 10/48, 39/48)
Neoplastic Effects	<u>Liver:</u> hepatocellular adenoma ([26/48], 25/48, 30/48, 34/48, 31/48, 26/48); hepatocellular carcinoma ([27/48], 15/48, 17/48, 20/48, 25/48, 27/48); hepatocellular adenoma or carcinoma (combined) ([41/48], 31/48, 35/48, 40/48, 42/48, 40/48)	None
Equivocal Findings	None	<u>Pancreas:</u> islets, adenoma ([0/48], 1/48, 0/48, 0/48, 0/48, 3/48)
Level of Evidence of Carcinogenic Activity	Some evidence	Equivocal evidence

Introduction

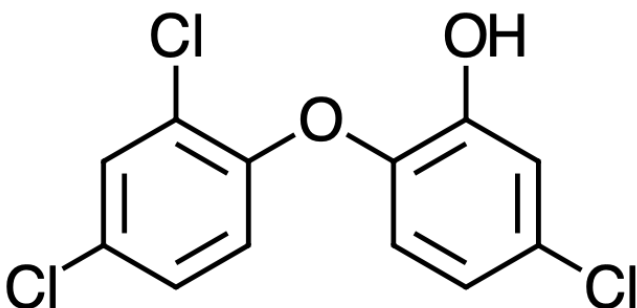


Figure 1. Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) (CASRN 3380-34-5; Chemical Formula: $C_{12}H_7Cl_3O_2$; Molecular Weight: 289.55)

Synonyms: 2,4,4-trichloro-2-hydroxydiphenyl ether, 2,4,4'-trichloro-2'-hydroxydiphenyl ether, trichloro-2'-hydroxydiphenyl ether.

Trade names: Irgasan, CH 3565, Irgasan CH 3565, Irgasan DP300, Ster-Zac, Tinosan AM110 Antimicrobial, Invasan DP 300R, Invasan DP 300 TEX, Irgaguard[®] B 1000, VIV-20, Irgacare MP, Lexol 300, Cloxifenolum, Aquasept, Gamophen, Vinyzene DP 7000, Vinyzene SB-30, Sanitized Brand, Microbanish R, Vikol THP, Ultra-Fresh, Microban Additive "B," AerisGuard, and Sapoderm.

Chemical and Physical Properties

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) (Figure 1) is a colorless to off-white crystalline powder with a slightly aromatic odor. It has a melting point of 55°C–57°C. Triclosan is slightly soluble in water (10 mg/L), moderately soluble in base (23.5 g/L in 0.1 N NaOH), and highly soluble (>1,000 g/L) in ethanol, acetone, propylene glycol, TWEEN[®] 20, benzene, and methyl Cellosolve[®].¹⁻³

Production, Use, and Human Exposure

Triclosan is synthesized by reacting 2,4,4'-trichloro-2'-methoxydiphenyl ether with aluminum chloride ($AlCl_3$). In the United States, the annual production of triclosan rose from 0.005– 0.2×10^6 kg in 1990 to >0.5 – 5×10^6 kg in 1998, the last year for which data are available.²

Triclosan is formulated as an antimicrobial active component in consumer care products, such as soaps, deodorants, toothpastes, and mouthwashes; household cleaners; and textiles, such as sportswear, bed clothes, shoes, and carpets. Triclosan preparations are also used to control the spread of methicillin-resistant *Staphylococcus aureus* in clinical settings and surgical scrubs, preoperative skin preparations, and sutures to prevent bacterial colonization of surgical wounds. Triclosan has been found in drinking water, surface water, wastewater, and environmental sediments, as well as in human breast milk, plasma, and urine.²⁻⁴

Biological and Toxicological Properties

Absorption, Distribution, Metabolism, and Excretion

After oral exposure, triclosan is primarily absorbed through the mucosal membranes of the oral cavity and gastrointestinal tract. Dermal contact results in absorption through the skin.² A dermal toxicokinetic study was conducted in male and female B6C3F1/N mice.⁵ When using 95%

ethanol as the vehicle, mice fitted with Elizabethan collars (to prevent oral ingestion) absorbed approximately 85% of the total dose administered. In mice that did not have Elizabethan collars, an additional 16%–23% was absorbed. At a dose of 10 mg triclosan/kg body weight (mg/kg), the absorption of triclosan was 6% greater in female mice compared to male mice. Likewise, the area under the curve (AUC_{0-∞}) and maximum observed concentration (C_{max}) of triclosan in the plasma and liver were greater in females than in males.⁵ After absorption, triclosan is readily metabolized to glucuronide and sulfate conjugates as well as 2,4-dichlorophenol, hydroxytriclosan, and 4-chlorocatechol.^{5; 6} Triclosan is excreted in the feces and urine. Rats and mice show predominantly biliary excretion into the feces, whereas guinea pigs excrete the majority of the dose via the kidney. In humans, urinary excretion is the major route of elimination.^{2; 3}

Carcinogenicity

The carcinogenicity of triclosan has been investigated in male and female CD-1 mice administered 0, 10, 30, 100, or 200 mg/kg/day in the diet for 18 months. Doses ≥ 30 mg/kg/day resulted in a significant increase in hepatocellular neoplasms in both males and females. Doses ≤ 10 mg/kg/day did not induce a tumorigenic response. The incidences of hepatocellular adenoma and adenoma or carcinoma (combined) showed a clear dose-response, with the incidences being higher in males than in females.³ A dermal triclosan carcinogenic study was conducted in which 0.5% and 1% triclosan in acetone (approximately equivalent to 20 and 40 mg/kg/application, respectively) was applied to the shaved intrascapular region of Swiss mice three times per week for 18 months.⁷ Specific details of the study were not provided; however, the authors reported that the findings in the triclosan-exposed mice were similar to those in the control mice and that triclosan was not found to be carcinogenic.

Study Rationale

There is potential for humans to be exposed to triclosan throughout their lifetime due to its extensive use in consumer care products and in clinical settings. Because there is very limited information regarding the dermal toxicity and carcinogenicity of triclosan, the U.S. Food and Drug Administration requested a dermal carcinogenicity study to conduct a safety assessment of triclosan-containing products.

Mice were used in this 2-year dermal carcinogenicity study because a previous oral carcinogenicity study in mice indicated that triclosan induced hepatocellular neoplasms.³ Furthermore, mice are the preferred species for dermal carcinogenicity studies under the U.S. Environmental Protection Agency's Health Effects Test Guidelines "*OPPTS 870.4300 Combined Chronic Toxicity/Carcinogenicity*,"⁸ and, historically, NTP dermal carcinogenicity assessments have been conducted in mice.

Materials and Methods

Procurement and Characterization

Triclosan

Triclosan was obtained from Alfa Aesar (Ward Hill, MA) in a single lot (5001A29X). Identity, purity, and stability analyses were conducted by the study laboratory at the U.S. Food and Drug Administration's National Center for Toxicological Research (NCTR, Jefferson, AR). Reports on analyses performed in support of the triclosan studies are on file at the National Institute of Environmental Health Sciences.

The identity of the test lot was evaluated using ^1H nuclear magnetic resonance (NMR) spectroscopy. The ^1H NMR spectrum was consistent with the structure of triclosan and identical to that of the reference standard (lot 1412854V, Sigma Aldrich, St. Louis, MO) (Appendix A). No resonances due to contaminants were present in the spectrum except those produced by water and solvent. The identity of lot 5001A29X was further confirmed using mass spectrometry (MS) after separation by high-performance liquid chromatography (HPLC) with diode array detection (PDA) (Table A-1). The MS spectrum was consistent with the structure of triclosan, which confirmed that the test lot corresponded to triclosan.

Purity evaluation was conducted by HPLC/PDA using a 1.0 mg/mL sample of the test lot dissolved in methanol (Table A-1). No impurities were identified. The overall purity of lot 5001A29X was estimated to be 99.9% by comparing the peak area with the reference standard, which was consistent with the 99.0% purity indicated by the manufacturer. The ^1H NMR analysis of the test article was consistent with this purity determination. Repeated purity evaluation conducted at the end of the study confirmed a purity of 99.9%.

The test article was received in amber jars and stored at room temperature.

Ethanol

The 95% ethanol dose vehicle was obtained from Decon Laboratories (King of Prussia, PA) in a single lot (2801G). The identity of ethanol was confirmed using ^1H NMR spectroscopy. The NMR spectrum was consistent with the structure of ethanol, and there was no evidence of contamination by other proton-containing compounds (Appendix A).

Purity evaluation of lot 2801G was conducted by the study laboratory using HPLC/PDA (Table A-1). The chromatograms did not show any ultraviolet chromophore-containing components above background, confirming a purity consistent with the 95% purity specified by the vendor. Batches of the ethanol dose vehicle were evaluated for the presence of triclosan before and after the study, and no triclosan was detected.

The ethanol dose vehicle was received in amber glass containers and stored at room temperature.

Preparation and Analysis of Dose Formulations

Dose formulations of triclosan (lot 5001A29X) in 95% ethanol were prepared by Priority One Services (Alexandria, VA) following protocols outlined in Appendix A. Dose formulations were

prepared at 1.25, 2.7, 5.8, and 12.5 mg/L (25 preparations; September 2013–August 2015) and were refrigerated, protected from light, and used within approximately 1 month of mixing.

Stability studies of the dose formulations stored at room (25°C) or refrigerated (2°C–8°C) temperatures were conducted using HPLC/PDA (Table A-1). The lowest (1.25 mg/mL) and highest (12.5 mg/mL) dose formulations were evaluated after 8 weeks at refrigerated temperatures or 4 weeks at room temperature. Stability for up to 56 days under refrigerated temperatures was confirmed. Additionally, the dose formulations were found to be homogeneous.

Analyses of preadministration dose formulations were conducted by the study laboratory using HPLC/PDA (Table A-1). All dose formulations were within 10% of the target concentrations.

Analysis of Triclosan in Experimental Background Materials

The animal feed (NTP-2000 Irradiated Rodent Diet) and drinking water (Millipore-filtered drinking water) were analyzed for the presence of background triclosan using ultra-performance liquid chromatography (UPLC) with MS (Table A-1). Animal feed samples were ground to a powder and extracted with acetonitrile. The acetonitrile supernatant was assayed by UPLC-MS and compared with feed samples spiked at 1 ppm triclosan. Although a prominent triclosan peak was observed in the spiked samples, the peak was not detectable in the unspiked samples, indicating that any possible background levels of triclosan in the feed were not present at or above 1 ppm. Additionally, peak areas observed for triclosan in the three drinking water samples were negligible in comparison with water samples spiked at 1 ppm triclosan, indicating that the drinking water triclosan levels were not present at or above 1 ppm.

Animal Source

Male and female B6C3F1/N mice were obtained from the National Toxicology Program (NTP) colony maintained by Taconic Biosciences, Inc. (Germantown, NY).

Animal Welfare

Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All animal studies were conducted in an animal facility accredited by AAALAC International. Studies were approved by the NCTR (Jefferson, AR) Animal Care and Use Committee and conducted in accordance with all relevant National Institutes of Health and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Two-year Study

Dose Selection Rationale

To formulate a scientific basis for the dose selection for the 2-year dermal carcinogenicity bioassay, a dermal subchronic study was conducted.⁴ Body weight changes and increases in the incidences of epidermal hyperplasia and parakeratosis at the site of application in the 27 mg triclosan/kg body weight/day (mg/kg/day) groups informed the selection of 12.5 mg/kg/day triclosan in ethanol as the high dose for male and female B6C3F1/N mice. The lowest dose

selected was 10-fold lower than the highest dose, and the intermediate doses were equally spaced between the highest and lowest doses, based on a $10^{(1/3)}$ dose spacing. Specifically, male and female B6C3F1/N mice were dermally administered 0, 1.25, 2.7, 5.8, or 12.5 mg/kg/day triclosan in 95% ethanol, 7 days per week for 2 years.

Study Design for Mice

Male and female B6C3F1/N mice were 3 weeks old upon receipt and were quarantined for 3 weeks before study start. At 5 weeks of age, mice were randomly assigned to one of six dose groups (n = 48 mice/sex/dose group). Randomization was stratified by body weight that produced similar group mean body weights using SAS software, Version 9.2 (Cary, NC). Starting at 6 weeks of age, mice were left untreated or dermally administered 0 (vehicle control group), 1.25, 2.7, 5.8, or 12.5 mg/kg/day triclosan for 2 years.

Approximately 24 hours before the first dermal administration of triclosan, the fur of each mouse was closely clipped on the dorsal surface from the shoulder region to the lumbar region using a Wahl Pocket Pro compact trimmer (Shelton, CT) with a size 40 blade. The clippers were battery operated and disposable; the blades were not changed. New clippers were used weekly (at the beginning of a clipping session) or as needed should the blade appear too dull during clipping. There were no instances when the clippers became hot. The mice groups were clipped in the following order: vehicle control, low dose, middle dose, and high dose. The untreated control animals were clipped after all other activities (i.e., weighing, clipping, and dosing animals; clinical observations; and cage changes) were completed for the vehicle control and dosed animals. Gloves were changed and the workstation was cleaned with disinfectant detergent and wiped with a 70% ethanol solution before clipping the untreated control mice. A separate set of clippers was used for the untreated control animals. The clipped area was rinsed with water, dried, and examined for nicks or breaks in the skin. The rinsing procedure was performed every time the animal was clipped. The site of application was re-clipped approximately once per week or as needed during the study. For animals with sores at the application site, care was taken to avoid clipping the fur in the areas containing sores to prevent further irritation to the area. Each animal in all six dose groups was clipped in this manner for the duration of the study. Any animal with abrasions or lesions in the clipped area was evaluated for possible exclusion from the study. No procedures were implemented to prevent incidental ingestion due to normal grooming behavior; however, animals were housed individually, and dorsal application was utilized to limit oral exposure. Elizabethan collars were not used due to the potential stress they would cause to the mice during the 2-year study.

All doses were administered in ethanol, at a dose volume of 1.0 mL/kg body weight; vehicle control animals received ethanol only and the untreated control group did not receive any dermal application of vehicle or test article. The animals were dosed in the following order: vehicle control, low dose, middle dose, and high dose (i.e., 0, 1.25, 2.7, 5.8, and 12.5 mg/kg/day). Dosing was conducted with a Hamilton Microlab 500 series pump, equipped with a Concord push-button hand pipettor/probe dispenser. The animals were placed on a grid, held by the base of their tails for dosing, and returned to their cages approximately 1 minute after the completion of dosing. The surface of the grid was cleaned if a dose was spilled as well as at the end of all dosing for the day. Gloves were changed between dose groups or more often if necessary. Dosing was completed for each mouse within 3 hours of the dosing time on the previous day. Untreated control animals were only handled while changing cages, clipping fur, weighing the

animals, and performing clinical observations. The activities for untreated control animals were performed last, and gloves were changed before these animals were handled.

Twenty-eight male and 28 female mice were randomly selected for parasite evaluation and gross observation of disease. The health of the mice was monitored during the study as described in Appendix C. Additional animals were evaluated for disease screening prior to quarantine release; however, data are not included here. All test results from quarantine and sentinel evaluations were negative.

Mice were housed individually. Feed and water were available ad libitum. Cages were changed twice weekly and rotated every 2 weeks. Racks were changed and rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is given in Appendix B.

Clinical Examinations and Pathology

In the 2-year study in mice, animals were observed twice daily for signs of morbidity and moribundity and were weighed before dermal administration of triclosan on day 1, weekly thereafter, and at study termination. Clinical observations were recorded weekly. Abnormal observations, including skin lesions, were recorded daily. Digital images of the site of application were taken monthly until the occurrence of a skin neoplasm, at which time digital images were taken weekly.

Complete necropsies and microscopic examinations were performed on all mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin except for eyes and testes, which were first fixed in modified Davidson's solution. Tissues were processed and trimmed, embedded in infiltrating media (Formula R[®]), sectioned at a thickness of approximately 5 µm, and stained with hematoxylin and eosin (H&E) for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by a quality assessment (QA) pathologist at a pathology laboratory independent of the study laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. A QA pathologist evaluated slides from all neoplasms and all potential target organs, which included the skin (site of application) and liver.

The QA report and the reviewed slides were submitted to the Pathology Working Group (PWG) coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and QA pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and QA pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the QA pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the

tissues without any knowledge of dose groups. When the PWG consensus diagnosis differed from that of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman⁹ and Boorman et al.¹⁰ For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was based generally on the guidelines of Brix et al.¹¹

Table 1. Experimental Design and Materials and Methods in the Two-year Dermal Study of Triclosan

Mice
Study Laboratory
National Center for Toxicological Research (Jefferson, AR)
Strain and Species
B6C3F1/N
Animal Source
Taconic Biosciences, Inc. (Germantown, NY)
Time Held Before Study
~3 weeks
Average Age When Study Began
6 weeks
Dates of First Dose
September 11–October 2, 2013
Duration of Dosing
2 years
Dates of Last Dose
September 6–29, 2015
Necropsy Dates
September 7–30, 2015
Average Age at Necropsy
2 years
Size of Study Groups
48/sex
Method of Distribution
Animals were distributed randomly into groups of approximately equal initial mean body weights
Animals per Cage
1
Method of Animal Identification
Cage card and tail tattoo

Mice

Diet

Irradiated NTP-2000 meal feed (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, changed weekly

Water

Millipore-filtered drinking water, available ad libitum

Cages

Standard National Center for Toxicological Research polycarbonate filtered bonnets cages (Allentown, Inc., Allentown, NJ), changed twice weekly, rotated every 2 weeks. Filtered bonnets changed every 2 weeks.

Bedding

Beta chip bedding (Northeastern Products Corp., Warrensburg, NY), changed with cage changes

Racks

Metal animal cage racks (Allentown, Inc., Allentown, NJ), rotated and changed every 2 weeks

Animal Room Environment

Temperature: 60°F–78.8°F

Relative humidity: 30%–90%

Room fluorescent light: 12 hours/day

Room air changes: 10–15/hour

Doses

Untreated, 0 (vehicle control group), 1.25, 2.7, 5.8, or 12.5 mg/kg/day in ethanol; 1 mL/kg dosing volume

Type and Frequency of Observation

Observed twice daily. Weighed before dermal administration on day 1, weekly thereafter, and at study termination. Clinical observations were recorded weekly. Abnormal observations, including skin lesions, were recorded daily. Digital images of the site of application were taken monthly until the occurrence of a skin neoplasm, at which time digital images were taken weekly.

Method of Euthanasia

Carbon dioxide

Necropsy

Necropsies were performed on all animals.

Histopathology

Complete histopathology was performed on all mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal cortex, adrenal medulla, blood vessel, bone marrow, brain (brain stem, cerebellum, and cerebrum), clitoral gland, epididymis, esophagus, eye, femur, gallbladder, Harderian gland, heart, large intestine (cecum, colon, and rectum), small intestine (duodenum, jejunum, and ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland with adjacent skin, nose, ovary, pancreas, pancreatic islets, parathyroid gland, pituitary gland, preputial gland, prostate, salivary glands, seminal vesicle, skin (control and site of application), spleen, stomach (forestomach and glandular), testes, thymus, thyroid gland, trachea, urinary bladder, and uterus.

Benchmark Dose Analysis

Benchmark doses (BMD) and the lower 95% confidence limits (BMDL) were calculated using U.S. Environmental Protection Agency Benchmark Dose Software (version 2.4.0.70; <https://www.epa.gov/bmds>). The calculations were conducted using gamma, logistic, log-

logistic, log-probit, multistage, probit, and Weibull models to fit the incidences of neoplasms and nonneoplastic lesions and the doses of triclosan administered dermally. The BMD₁₀ and BMDL₁₀ were defined as the dose that caused a 10% excess risk of the specified adverse effect over that observed in the vehicle control group and as the 95% lower bound on the BMD corresponding to a 10% extra risk, respectively.

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier¹² and is presented graphically. Animals surviving to the end of the observation period are treated as censored observations, as are animals dying from unnatural causes within the observation period. Animals dying from natural causes are included in analyses and are treated as uncensored observations. For the 2-year mouse study, dose-related trends are identified with Tarone's life-table test,¹³ and pairwise dose-related effects are assessed using Cox's method.¹⁴ All reported p values for the survival analyses are two-sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented as the numbers of animals bearing such lesions at a specific anatomic site. For calculation of incidence rates, the denominator for most neoplasms and all nonneoplastic lesions is the number of animals for which the site was examined microscopically. When macroscopic examination was required to detect neoplasms in certain tissues (e.g., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, however, the denominator consists of the number of animals that had a gross abnormality. When neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominator consists of the number of animals on which a necropsy was performed. Additional study data also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal euthanasia.

Analysis of Neoplasm and Nonneoplastic Lesion Incidence

Statistical analyses of neoplasm and nonneoplastic lesion incidence considered two features of the data. Some animals did not survive the entire 2 years of the study, so survival differences between groups had to be considered.

The Poly-k test¹⁵⁻¹⁷ was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to account for survival differences. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is 1 if the animal had a lesion at that site or if it survived until terminal euthanasia; if the animal died before terminal euthanasia and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only on the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time.¹⁵ Unless otherwise specified, a value of $k = 3$ was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier¹⁵ after an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control Fischer 344 rats and B6C3F1 mice.¹⁸ Bailer and Portier¹⁵ showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams.¹⁹ Poly-3 tests used the continuity correction described by Nam.²⁰

Tests of significance included pairwise comparisons of each dosed group with the vehicle control group, a pairwise comparison of the vehicle control group with the untreated control group, and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence and reported p values are one-sided. The significance of a lower incidence or negative trend in lesions is approximated as $1-p$ with the letter N added (e.g., $p = 0.99$ is presented as $p = 0.01N$).

Analysis of Continuous Variables

Each animal was weighed weekly starting at approximately PND 48; however, animals were not weighed at precisely the same time point relative to birth. Therefore, prior to analysis, locally estimated scatterplot smoothing (LOESS) was performed on the body weight data to rasterize to a set of common time points and reduce their number. The statistical model for the analysis of body weight was a repeated-measures, mixed-effects analysis of variance (ANOVA) model.²¹ A heterogeneous autoregressive covariance structure was used to account for body weight correlation across times within an animal and for the increasing variability in body weight as the body weights get larger. Comparisons to the vehicle control group were corrected using the Dunnett-Hsu method.²² To address considerable variability in body weights in the second year of the study and to consider any bias introduced during this time, an ANOVA was run on the full 2-year data record and also on a reduced 1-year data record (PND 50–386), during which minimal animal loss and body weight variations were observed.

Historical Control Data

The concurrent vehicle control group is the most valid comparison to the dosed groups and is the only control group analyzed statistically in NTP bioassays. Historical control data are often helpful in interpreting potential dose-related effects, however, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical control data must be generally similar. Significant factors affecting the background incidence of neoplasms at a variety of sites are diet, sex, strain/stock, and route of exposure. The NTP historical control database contains all 2-year studies for each species, sex, and strain/stock with histopathology findings in control animals completed within the most recent 5-year period²³⁻²⁵ for comparison across multiple technical reports. In general, the historical control data for a given study includes studies using the same route of administration, and the overall incidence of neoplasms in controls for all routes of administration are included for comparison.

Quality Assurance Methods

The 2-year study was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice Regulations.²⁶ In addition, a draft of this NTP Technical Report was audited by an independent QA contractor against the study protocol and report. The audit findings were reviewed and assessed by Division of Translational Toxicology and National Center for Toxicological Research staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

Results

Data Availability

All study data were evaluated. Data relevant for evaluating toxicological findings are presented here. All study data are available in the National Toxicology Program (NTP) Chemical Effects in Biological Systems (CEBS) database: <https://doi.org/10.22427/NTP-DATA-TR-604>.²⁷

Mice

Two-year Study

Estimates of survival probabilities for mice are shown in Table 2 and in the Kaplan-Meier survival curves (Figure 2). The survival of all dosed groups of male and female mice was similar to that of the vehicle control group.

Table 2. Summary of Survival of Male and Female Mice in the Two-year Dermal Study of Triclosan

	Untreated Control	Vehicle Control	1.25 mg/kg/day	2.7 mg/kg/day	5.8 mg/kg/day	12.5 mg/kg/day
Male						
Animals Initially in Study	48	48	48	48	48	48
Moribund	14	11	18	12	19	17
Accidental Deaths	0	1	0	0	0	0
Natural Deaths	3	1	0	2	1	0
Animals Surviving to Study Termination	31	35	30	34	28	31
Percent Probability of Survival at Study Termination ^a	64.6	74.5	62.5	70.8	58.3	64.6
Survival (Days) ^b	676.7 ± 11.7	682.7 ± 16.6	671.8 ± 14.2	695.2 ± 9.8	671.2 ± 13.4	671.5 ± 15.0
Survival Analysis ^c	– ^d	p = 0.527	p = 0.259	p = 0.876	p = 0.138	p = 0.353
Female						
Animals Initially in Study	48	48	48	48	48	48
Moribund	15	12	18	17	8	11
Natural Deaths	0	1	1	0	3	2
Animals Surviving to Study Termination	33	35	29	31	37	35
Percent Probability of Survival at Study Termination	68.8	72.9	60.4	64.6	77.1	72.9
Survival (Days)	691.6 ± 10.0	694.4 ± 10.2	674.7 ± 13.4	691.7 ± 9.3	678.1 ± 18.2	696.4 ± 9.3
Survival Analysis	–	p = 0.445N	p = 0.248	p = 0.543	p = 0.902N	p = 1.000N

^aKaplan-Meier determinations.

^bMean of all deaths (uncensored, censored, and study termination) ± standard error.

^cThe result of the Tarone trend test is in the vehicle control group column, the results of the Cox proportional hazards pairwise comparisons to the vehicle control group are in the dosed group columns. A negative trend or lower mortality in a dose group is indicated by N.

^dNo trend or pairwise statistical tests were performed to compare the untreated control group to the vehicle control group.

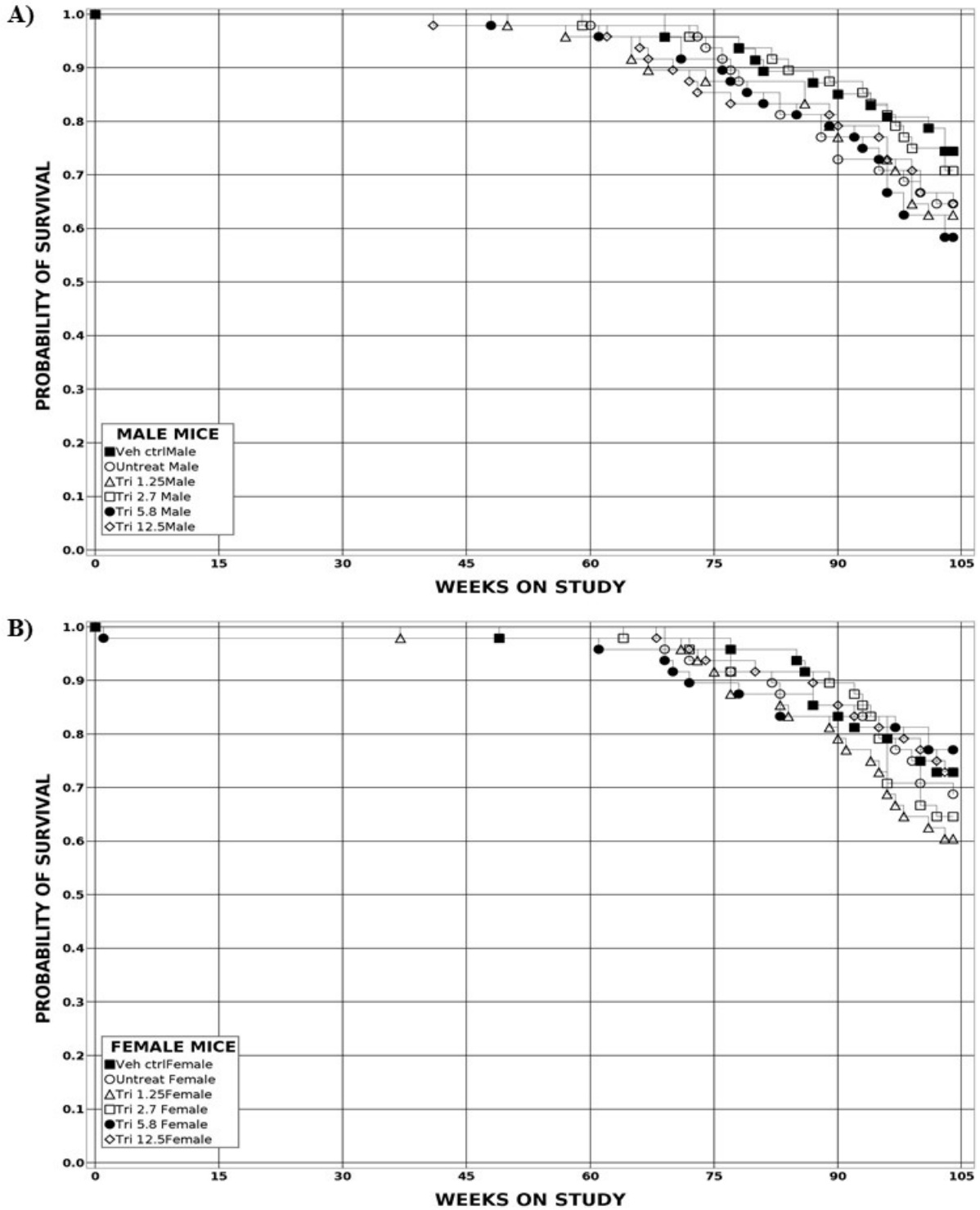


Figure 2. Kaplan-Meier Survival Curves for Male and Female Mice in the Two-year Dermal Study of Triclosan

Survival curves are shown for (A) males and (B) females.

At the site of application, minimal to mild skin lesions, including cuts, scratches, dark pigmentation, dry skin, flaking, sore, and tissue mass, were observed (Appendix E). Scratches were considered to be dose-related in both sexes. Dry skin and flaking either did not occur or occurred at a low incidence except in the 12.5 mg/kg/day male and female groups. Dark pigmentation was considered to be related to vehicle administration, and all other lesions were not considered to be related to vehicle or triclosan administration. These lesions were not considered sufficiently severe to require early termination of any of the mice in the study.

In male mice, dermal administration of triclosan over the full 2 years of the study caused a negative trend in body weights, with the decrease being significant in the 12.5 mg/kg/day group relative to the vehicle control group (Table 3; Figure 3). Compared to the vehicle control group, the decreases in the body weights of male mice dosed with 12.5 mg/kg/day never exceeded 7%. In female mice, dermal administration of triclosan over the full 2 years of the study caused a negative trend in body weights, with the decrease being significant in the 12.5 mg/kg/day group relative to the vehicle control group (Table 4; Figure 3). Compared to the vehicle control group, the decreases in the body weights of female mice dosed with 12.5 mg/kg/day never exceeded 8%.

Table 3. Summary of Survival and Body Weights of Male Mice in the Two-year Dermal Study of Triclosan

Study Week ^a	Untreated Control		Vehicle Control			1.25 mg/kg/day			2.7 mg/kg/day			5.8 mg/kg/day			12.5 mg/kg/day		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^b	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^c	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^c	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^c	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^c	No. of Survivors
1	23.7	48	23.0	97	48	23.6	102	48	23.2	101	48	23.4	102	48	23.4	101	48
4	27.2	48	26.9	99	48	27.3	101	48	26.8	99	48	27.0	100	48	26.8	100	48
8	30.2	48	30.3	100	47	30.2	100	48	29.9	99	48	30.6	101	48	30.4	101	48
12	33.7	48	33.2	98	47	33.4	101	48	32.9	99	48	34.7	104	48	33.6	101	48
16	37.0	48	35.6	96	47	36.2	102	48	35.9	101	48	36.9	104	48	35.7	101	48
20	38.4	48	37.8	98	47	38.6	102	48	38.0	101	48	38.6	102	48	37.5	99	48
24	41.4	48	40.8	99	47	41.7	102	48	40.6	100	48	41.7	102	48	40.5	99	48
28	43.9	48	42.9	98	47	43.4	101	48	42.6	99	48	43.6	102	48	42.2	99	48
32	45.5	48	44.5	98	47	45.3	102	48	43.9	99	48	45.3	102	48	43.3	98	48
36	47.1	48	45.1	96	47	46.7	104	48	45.1	100	48	46.1	102	48	44.9	100	48
40	48.7	48	46.6	96	47	48.1	103	48	46.2	99	48	47.6	102	48	45.6	98	47
44	49.1	48	48.3	98	47	49.1	102	48	47.4	98	48	49.1	102	48	47.5	98	47
48	49.7	48	49.1	99	47	49.4	101	48	47.5	97	48	50.1	102	47	48.1	98	47
52	50.3	48	49.1	98	47	49.6	101	47	47.7	97	48	50.0	102	47	48.1	98	47
56	50.4	48	49.0	97	47	49.9	102	46	48.3	99	48	50.6	103	47	48.3	99	47
60	50.3	47	49.2	98	47	49.9	101	46	48.5	99	47	50.1	102	46	47.6	97	47
64	50.8	47	49.8	98	47	50.1	101	44	49.1	99	47	50.9	102	46	47.7	96	46
68	50.8	47	50.4	99	46	50.5	100	43	49.4	98	47	50.6	100	46	47.7	95	44
72	51.4	46	50.9	99	45	50.7	100	43	49.8	98	46	51.1	100	44	49.2	97	42
76	51.0	43	50.4	99	45	50.8	101	42	50.3	100	46	51.5	102	42	49.2	98	40
80	50.9	41	51.1	100	43	50.7	99	42	50.1	98	45	51.5	101	40	48.7	95	40
84	51.5	39	51.6	100	42	50.9	99	42	50.4	98	43	51.6	100	39	48.7	94	40
88	51.3	36	51.8	101	41	50.5	96	39	49.7	97	42	50.5	97	38	48.7	94	39

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Study Week ^a	Untreated Control		Vehicle Control			1.25 mg/kg/day			2.7 mg/kg/day			5.8 mg/kg/day			12.5 mg/kg/day		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^b	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^c	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^c	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^c	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^c	No. of Survivors
92	50.6	35	51.3	101	40	49.4	96	37	49.0	96	42	49.6	97	36	47.8	93	38
96	50.4	33	50.6	100	38	48.8	96	35	49.9	99	39	49.9	99	32	47.3	93	35
100	49.9	32	50.3	101	37	48.7	97	30	49.5	98	36	49.5	98	30	47.6	95	32
Mean for Weeks 1–104 ^{c,d}	44.6		44.2**	99		44.2	100		43.3	98		44.4	100		42.7**	97	

Statistical significance for the untreated control group or a dosed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

**Statistically significant at $p \leq 0.01$.

^aWeight (% of controls) is given as a percentage of the untreated control.

^bWeight (% of controls) is given as a percentage of the vehicle control.

^cStatistical analysis performed using repeated-measures mixed-effects ANOVA (trend) and the Dunnett-Hsu (pairwise) tests.

^dTrend analysis was also performed on the vehicle control group and on all exposed groups using a reduced 1-year data record of the mean body weights for postnatal day 50–386.

No statistically significant findings were noted at $p \leq 0.05$.

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Table 4. Summary of Survival and Body Weights of Female Mice in the Two-year Dermal Study of Triclosan

Study Week ^a	Untreated Control		Vehicle Control			1.25 mg/kg/day			2.7 mg/kg/day			5.8 mg/kg/day			12.5 mg/kg/day		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^a	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^b	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^b	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^b	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^b	No. of Survivors
1	19.7	48	19.5	99	48	19.6	101	48	19.6	101	48	19.4	100	47	19.8	101	48
4	23.1	48	23.2	101	48	23.5	101	48	23.3	100	48	23.1	99	47	23.4	101	48
8	25.8	48	26.2	102	48	26.3	100	48	26.2	100	48	25.9	99	47	25.8	98	48
12	29.1	48	29.2	100	48	29.3	100	48	29.2	100	48	29.2	100	47	28.4	97	48
16	31.7	48	32.1	101	48	32.1	100	48	31.9	99	48	31.4	98	47	30.2	94	48
20	33.2	48	33.9	102	48	33.9	100	48	33.6	99	48	33.0	98	47	31.7	94	48
24	36.8	48	36.6	99	48	36.3	99	48	36.1	99	48	35.4	97	47	34.5	94	48
28	39.3	48	39.0	99	48	38.4	98	48	38.4	98	48	37.8	97	47	36.8	94	48
32	42.3	48	41.1	97	48	40.7	99	48	40.3	98	48	39.2	95	47	38.1	93	48
36	44.6	48	42.3	95	48	42.3	100	47	41.5	98	48	40.1	95	47	39.8	94	48
40	45.9	48	43.8	95	48	44.1	101	47	43.3	99	48	42.2	96	47	41.1	94	48
44	47.8	48	46.3	97	48	46.0	99	47	45.4	98	48	44.2	95	47	42.8	92	48
48	49.3	48	47.4	96	48	46.9	99	47	46.0	97	48	45.2	96	47	43.9	93	48
52	49.7	48	48.4	97	47	47.4	98	47	46.7	97	48	45.9	96	47	43.9	92	48
56	49.9	48	48.4	97	47	48.1	99	47	47.0	98	48	46.3	97	47	44.7	93	48
60	49.2	48	48.6	99	47	48.4	100	47	47.0	97	48	46.8	97	46	44.8	93	48
64	50.0	48	49.5	99	47	48.9	99	47	48.0	97	47	48.2	97	46	45.4	92	48
68	50.4	46	50.1	99	47	49.8	99	47	48.2	96	47	48.7	97	45	46.4	93	47
72	50.6	45	51.5	102	47	49.9	97	46	49.0	95	46	49.7	97	43	47.2	92	46
76	51.4	44	50.8	99	47	49.9	98	42	49.1	97	44	49.3	97	43	47.3	93	45
80	50.5	44	51.4	102	46	49.4	96	42	49.0	95	44	48.9	95	42	47.9	93	44
84	50.3	42	51.7	103	45	50.0	97	40	49.6	96	44	49.9	97	40	47.7	92	44
88	50.6	41	51.6	102	41	49.8	97	39	49.1	95	43	49.1	95	40	47.4	92	43

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Study Week ^a	Untreated Control		Vehicle Control			1.25 mg/kg/day			2.7 mg/kg/day			5.8 mg/kg/day			12.5 mg/kg/day		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^a	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^b	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^b	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^b	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls) ^b	No. of Survivors
92	49.7	40	51.0	103	39	48.8	96	37	48.1	94	41	48.0	94	40	47.7	94	40
96	48.6	38	49.7	102	38	46.9	94	33	46.8	94	34	48.2	97	39	46.9	94	39
100	47.7	34	48.8	102	36	46.9	96	30	45.6	93	32	47.1	97	37	46.1	94	37
Mean for Weeks 1–104 ^{c,d}	42.6		42.3***	99		41.6	98		41.1	97		40.7	96		39.4***	93	

Statistical significance for the untreated control group or a dosed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

***Statistically significant at $p \leq 0.001$.

^aWeight (% of controls) is given as a percentage of the untreated control.

^bWeight (% of controls) is given as a percentage of the vehicle control.

^cStatistical analysis performed using mixed-effects ANOVA (trend) and the Dunnett-Hsu (pairwise) tests.

^dTrend analysis was also performed on the vehicle control group and on all exposed groups using a reduced 1-year data record of the mean body weights for postnatal day 50–386. Statistically significant findings were noted at $p \leq 0.001$ for the trend and pairwise comparison in the 12.5 mg/kg/day group.

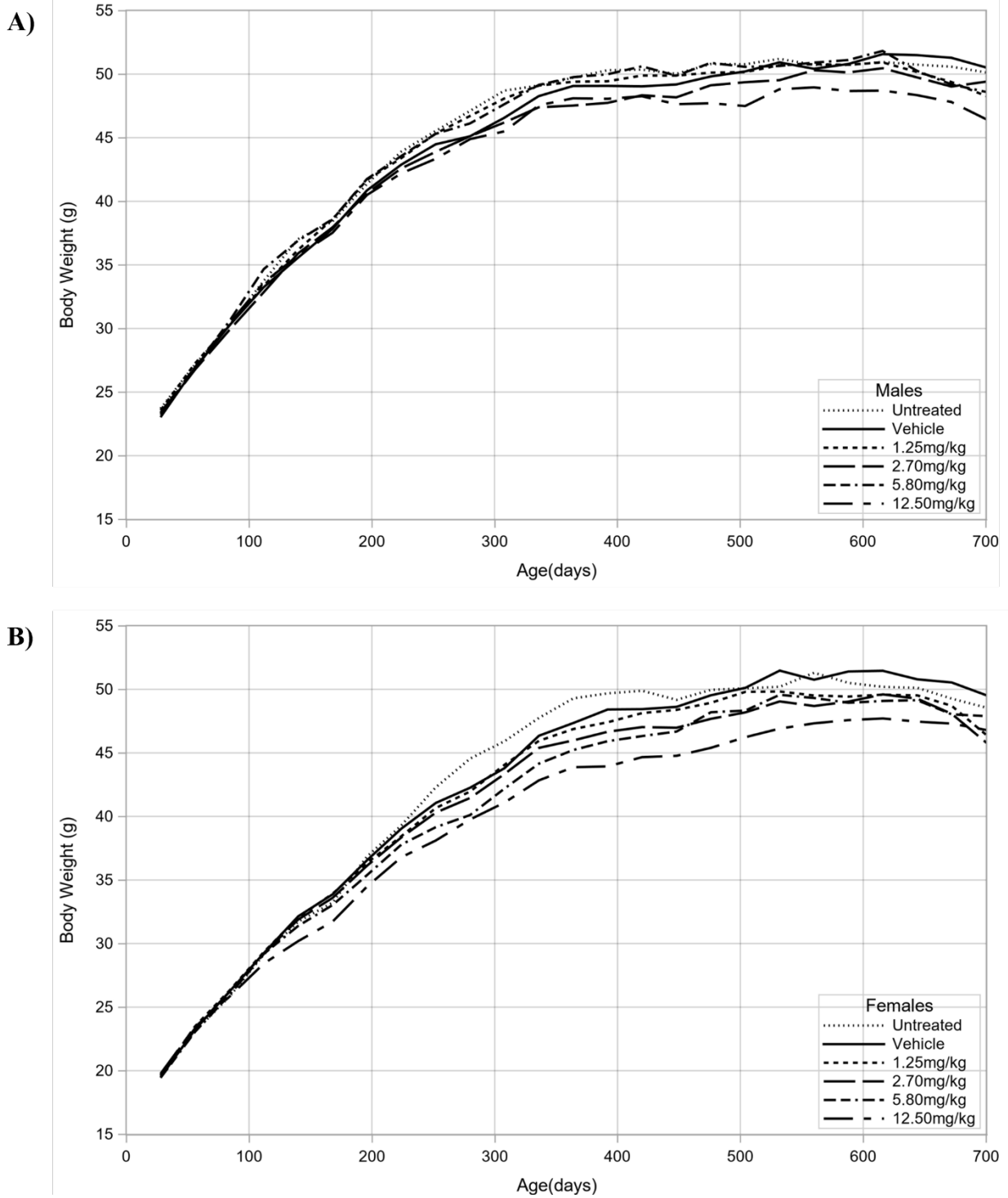


Figure 3. Growth Curves for Male and Female Mice in the Two-year Dermal Study of Triclosan

Growth curves are shown for (A) males and (B) females.

Histopathology

This section describes the statistically significant or biologically noteworthy changes in the incidence of neoplasms and/or nonneoplastic lesions of the liver, pancreas, and skin. The appropriate statistical comparison is to the vehicle control animals, which were subjected to the same daily stress of handling and dosing as the triclosan-dosed groups. Data from the untreated control animals are also reported.

Liver: In male mice, triclosan administration resulted in positive trends in the incidences of hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined), with the increase being significant in the ≥ 5.8 and ≥ 2.7 mg/kg/day groups, respectively, compared to the vehicle control group (Table 5). The incidence of hepatocellular carcinomas in the untreated control males was higher than that in the vehicle control males (Table 5). In female mice, there were no significant increases in the incidences of hepatocellular adenoma, hepatocellular carcinoma, or hepatocellular adenoma or carcinoma (combined). The multiplicity of hepatocellular neoplasms is presented in Table 5. No exposure-related nonneoplastic lesions were observed in the liver.

Table 5. Incidences of Neoplasms of the Liver in Male and Female Mice in the Two-year Dermal Study of Triclosan

	Untreated Control	Vehicle Control	1.25 mg/kg/day	2.7 mg/kg/day	5.8 mg/kg/day	12.5 mg/kg/day
n^a	48	48	48	48	48	48
Male						
Hepatocellular Adenoma, Multiple ^{b,c}	16 (33%)	16 (33%)	21 (44%)	23 (48%)	17 (35%)	17 (35%)
Hepatocellular Adenoma (Includes Multiple) ^d						
Overall rate ^e	26/48 (54%)	25/48 (52%)	30/48 (63%)	34/48 (71%)	31/48 (65%)	26/48 (54%)
Adjusted rate ^f	61.1%	56.8%	71.2%	75.1%	72.4%	59.2%
Terminal rate ^g	20/31 (65%)	21/35 (60%)	23/30 (77%)	27/34 (79%)	23/28 (82%)	17/31 (55%)
First incidence (days)	513	478	597	407	492	282
Poly-3 test ^h	–	p = 0.328N	p = 0.113	p = 0.047	p = 0.088	p = 0.495
Poly-3 test (controls) ⁱ	–	p = 0.423N	–	–	–	–
Hepatocellular Carcinoma, Multiple	3 (6%)	1 (2%)	6 (13%)	4 (8%)	6 (13%)	6 (13%)
Hepatocellular Carcinoma (Includes Multiple) ^j						
Overall rate	27/48 (56%)	15/48 (31%)	17/48 (35%)	20/48 (42%)	25/48 (52%)	27/48 (56%)
Adjusted rate	57%	32.5%	38.8%	43.0%	54.4%	59.5%
Terminal rate	12/31 (39%)	7/35 (20%)	8/30 (27%)	11/34 (32%)	11/28 (39%)	14/31 (45%)
First incidence (days)	417	478	468	407	336	462
Poly-3 test	–	p = 0.003	p = 0.343	p = 0.204	p = 0.025	p = 0.007
Poly-3 test (controls)	–	p = 0.013N	–	–	–	–
Hepatocellular Adenoma or Carcinoma (Combined) ^k						
Overall rate	41/48 (85%)	31/48 (65%)	35/48 (73%)	40/48 (83%)	42/48 (88%)	40/48 (83%)
Adjusted rate	85.4%	67.1%	78.8%	84.9%	89.0%	84.7%
Terminal rate	24/31 (77%)	22/35 (63%)	23/30 (77%)	28/34 (82%)	24/28 (86%)	24/31 (77%)
First incidence (days)	417	478	468	407	336	282
Poly-3 test	–	p = 0.044	p = 0.150	p = 0.034	p = 0.008	p = 0.036
Poly-3 test (controls)	–	p = 0.029N	–	–	–	–

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	Untreated Control	Vehicle Control	1.25 mg/kg/day	2.7 mg/kg/day	5.8 mg/kg/day	12.5 mg/kg/day
Female						
Hepatocellular Adenoma, Multiple	11 (23%)	5 (10%)	5 (10%)	8 (17%)	9 (19%)	5 (10%)
Hepatocellular Adenoma (Includes Multiple) ^l						
Overall rate	22/48 (46%)	17/48 (35%)	13/48 (27%)	17/48 (35%)	18/48 (38%)	17/48 (35%)
Adjusted rate	49.2%	38.3%	31.1%	39.4%	41.3%	37.8%
Terminal rate	18/33 (55%)	13/35 (37%)	9/29 (31%)	13/31 (42%)	13/37 (35%)	12/35 (34%)
First incidence (days)	483	590	533	664	580	475
Poly-3 test	–	p = 0.440	p = 0.318N	p = 0.545	p = 0.470	p = 0.566N
Poly-3 test (controls)	–	p = 0.203N	–	–	–	–
Hepatocellular Carcinoma, Multiple	2 (4%)	0 (0%)	0 (0%)	1 (2%)	2 (4%)	0 (0%)
Hepatocellular Carcinoma (Includes Multiple) ^m						
Overall rate	12/48 (25%)	10/48 (21%)	6/48 (13%)	9/48 (19%)	8/48 (17%)	7/48 (15%)
Adjusted rate	26.7%	22.6%	14.4%	20.6%	18.2%	15.7%
Terminal rate	7/33 (21%)	6/35 (17%)	2/29 (7%)	6/31 (19%)	4/37 (11%)	4/35 (11%)
First incidence (days)	477	596	533	535	484	475
Poly-3 test	–	p = 0.330N	p = 0.241N	p = 0.514N	p = 0.405N	p = 0.290N
Poly-3 test (controls)	–	p = 0.418N	–	–	–	–
Hepatocellular Adenoma or Carcinoma (Combined) ⁿ						
Overall rate	25/48 (52%)	22/48 (46%)	18/48 (38%)	21/48 (44%)	21/48 (44%)	20/48 (42%)
Adjusted rate	54.6%	48.8%	42.3%	47.7%	46.8%	44.4%
Terminal rate	18/33 (55%)	16/35 (46%)	11/29 (38%)	15/31 (48%)	14/37 (38%)	15/35 (43%)
First incidence (days)	477	590	533	535	484	475
Poly-3 test	–	p = 0.450N	p = 0.346N	p = 0.541N	p = 0.506N	p = 0.418N
Poly-3 test (controls)	–	p = 0.366N	–	–	–	–

^aNumber of animals with tissue examined microscopically.

^bNumber of animals with lesion.

^cNo statistical analyses were performed on multiplicity data.

^dHistorical control incidence for all routes of 2-year studies (mean ± standard deviation): 398/789 (50.04 ± 9.88%); range: 34% to 70%.

^eNumber of animals with neoplasm/number of animals necropsied.

^fPoly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.

^gObserved incidence at study termination.

^hBeneath the vehicle control incidence is the p value associated with the trend test. Beneath the dosed group incidence is the p value corresponding to pairwise comparisons between the vehicle control group and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach study termination. A negative trend or a lower incidence in a dose group is indicated by N.

ⁱBeneath the vehicle control incidence is the p value corresponding to a pairwise comparison between the vehicle control group and the untreated control group. A lower incidence in the vehicle control group is indicated by N.

^jHistorical control incidence: 201/789 (25.16% ± 9.06%); range: 10% to 42%.

^kHistorical control incidence: 513/789 (64.5% ± 10.59%); range: 46% to 80%.

^lHistorical control incidence: 150/839 (17.71% ± 7.01%); range: 6% to 28%.

^mHistorical control incidence: 71/839 (8.44% ± 18%); range: 2% to 20%.

ⁿHistorical control incidence: 203/849 (23.88% ± 8.96%); range: 8% to 40%.

Pancreas: Female mice dosed with triclosan had a positive trend in the incidence of pancreatic islet adenoma; however, the increased incidence did not reach statistical significance in pairwise comparisons in dosed groups compared to the vehicle control group (Table 6). Neither pancreatic islet hyperplasia nor adenoma was observed in untreated female mice. Pancreatic islet carcinoma was observed in one female in both the untreated and vehicle control groups.

Pancreatic islet hyperplasia was characterized by an increase in the size of islets and islet cell numbers featuring a multifocal to diffuse distribution. The outline of hyperplastic islets was sometimes irregular with no compression of the surrounding acinar tissue. Islet cells were frequently hypertrophic but uniform. In contrast, islet cell adenomas were larger and well-circumscribed with compression of the peripheral tissue. Cellular growth patterns within the neoplasm ranged from sheets to nests or ribbons and pale staining.

Table 6. Incidences of Neoplasms and Nonneoplastic Lesions of the Pancreas in Female Mice in the Two-year Dermal Study of Triclosan

	Untreated Control	Vehicle Control	1.25 mg/kg/day	2.7 mg/kg/day	5.8 mg/kg/day	12.5 mg/kg/day
n^a	48	48	48	48	48	48
Islets, Hyperplasia ^b	0	1 (2.0) ^c	0	1 (2.0)	0	1 (2.0)
Islets, Adenoma ^d						
Overall rate ^e	0/48 (0%)	1/48 (2%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	3/48 (6%)
Adjusted rate ^f	0.0%	2.3%	0.0%	0.0%	0.0%	6.9%
Terminal rate ^g	0/33 (0.0%)	0/35 (0%)	0/29 (0%)	0/31 (0%)	0/37 (0%)	2/35 (6%)
First incidence (days) ^h	–	590	–	–	–	663
Poly-3 test ⁱ	–	p = 0.033 ^j	p = 0.515N	p = 0.505N	p = 0.507N	p = 0.305
Islets, Carcinoma ^k	1	1	0	0	0	0
Islets, Adenoma or Carcinoma (Combined) ^l						
Overall rate	1/48 (2%)	2/48 (4%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	3/48 (6%)
Adjusted rate	2.4%	4.5%	0.0%	0.0%	0.0%	6.9%
Terminal rate	1/33 (3%)	0/35 (0%)	0/29 (0%)	0/31 (0%)	0/37 (0%)	2/35 (6%)
First incidence (days)	726	590	–	–	–	663
Poly-3 test	–	p = 0.121	p = 0.257N	p = 0.245N	p = 0.247N	p = 0.495
Poly-3 test (controls) ^m	–	p = 0.511	–	–	–	–

^aNumber of animals with tissue examined microscopically.

^bNumber of animals with lesion.

^cAverage severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

^dHistorical control incidence for all routes of 2-year studies (mean ± standard deviation): 3/832 (0.38% ± 0.81%); range: 0% to 2%.

^eNumber of animals with neoplasm/number of animals necropsied.

^fPoly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.

^gObserved incidence at terminal euthanasia.

^hNot applicable if there were no neoplasms in group.

ⁱBeneath the vehicle control incidence is the p value associated with the trend test. Beneath the dosed group incidence is the p value corresponding to pairwise comparisons between the vehicle control group and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach study termination. A negative trend or a lower incidence in a dose group is indicated by N.

^jA Poly-3 analysis was conducted only if the incidence in one of the groups was ≥4% and was therefore not run to compare the vehicle control group with the untreated control group.

^kHistorical control incidence: 1/832 (0.07% ± 0.29%); range: 0% to 1%.

^lHistorical control incidence: 4/832 (0.45% ± 0.82%); range: 0% to 2%.

^mBeneath the vehicle control incidence is the p value corresponding to a pairwise comparison between the vehicle control group and the untreated control group.

Skin: Dose-related minimal to mild skin lesions were observed at the site of application in male and female mice. Male mice dosed with triclosan had a positive trend in the incidences of hyperplasia, suppurative inflammation, and ulceration of the epidermis at the site of application (Table 7). The incidence of epidermal hyperplasia became significant at 2.7 mg/kg/day and was present in approximately 90% of male mice at 12.5 mg/kg/day. The incidences of epidermal

suppurative inflammation and ulceration were significantly increased at 12.5 mg/kg/day. Female mice dosed with triclosan had a positive trend in the incidence of hyperplasia of the epidermis at the site of application (Table 7). The incidence of epidermal hyperplasia became significant at 1.25 mg/kg/day and was present in approximately 80% of female mice at 12.5 mg/kg/day.

Epidermal hyperplasia was characterized by a thickening of the squamous epithelium due to increased layers of nucleated cells in the epidermis, with one or two cell layers considered as normal, three or four layers as minimal hyperplasia, five or six layers as mild hyperplasia, seven or eight layers as moderate hyperplasia, and more than eight layers as marked hyperplasia. Epidermal suppurative inflammation exhibited a predominant neutrophilic infiltrate. Epidermal ulceration was defined as complete loss of the epidermis with an overlying serocellular crust.

Table 7. Incidences of Nonneoplastic Lesions of the Skin at the Site of Application in Male and Female Mice in the Two-year Dermal Study of Triclosan

	Untreated Control	Vehicle Control	1.25 mg/kg/day	2.7 mg/kg/day	5.8 mg/kg/day	12.5 mg/kg/day
n^a	48	48	48	48	48	48
Male						
Epidermis, Hyperplasia ^b	2 (2.0) ^c	1** (1.0)	3 (2.3)	20** (1.2)	17** (1.2)	43** (1.4)
Epidermis, Inflammation, Suppurative	2 (2.5)	0**	3 (2.7)	2 (2.0)	4 (1.5)	9** (2.0)
Epidermis, Ulcer	2 (2.0)	0*	2 (3.0)	1 (3.0)	2 (1.5)	5* (2.4)
Female						
Epidermis, Hyperplasia	1 (1.0)	0**	5* (1.8)	7** (1.1)	10** (1.2)	39** (1.2)

Statistical significance for a dosed group indicates a significant pairwise test compared to the vehicle control group. Statistical significance for the vehicle control group indicates a significant trend test.

*Statistically significant at $p \leq 0.05$ by the Poly-3 test; ** $p \leq 0.01$.

^aNumber of animals examined microscopically.

^bNumber of animals with lesion.

^cAverage severity grade of observed lesion in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked.

Other tissues: Male mice had positive trends in the incidences of histocyte infiltration of the lung and lymphoid tissue hyperplasia of the small intestine (Appendix E). Significantly increased incidences of lymphoid hyperplasia of the mandibular lymph node and lymphocyte infiltration of the urinary bladder were observed at 5.8 mg/kg/day. Suppurative inflammation of the preputial gland was also observed at 1.25 mg/kg/day. There were no differences in nonneoplastic lesions between the vehicle control group and the untreated control group. Female mice also had positive trends in the incidences of angiectasis and pars distalis hyperplasia of the pituitary gland, with the incidence of angiectasis becoming significant at 12.5 mg/kg/day. Pancreatic ductal dilatation also had a positive trend. Significantly increased incidences of lymphocytic infiltration of the Harderian gland and duct dilatation of the clitoral gland were observed at 12.5 mg/kg/day and 1.25 mg/kg/day, respectively. Except for lymphocytic infiltration of the Harderian gland, there were no differences in nonneoplastic lesions between the vehicle control group and the untreated control group (Appendix E).

Benchmark Dose Analysis

Benchmark dose (BMD) modeling was conducted on neoplasms (hepatocellular carcinoma in male mice and pancreatic islet adenoma in female mice) and nonneoplastic lesions (hyperplasia, suppurative inflammation, and ulceration of the epidermis in male mice and hyperplasia of the epidermis in female mice) (Table 8). Additional nonneoplastic lesions used for BMD modeling are presented in Appendix D. The 95% lower bound on the BMD corresponding to a 10% extra risk (BMDL₁₀) for hepatocellular carcinoma in male mice ranged from 1.1 to 2.4 mg/kg/day, whereas the BMDL₁₀ for pancreatic islet adenoma in female mice ranged from 11.7 to 12.3 mg/kg/day. In both male and female mice, the most sensitive nonneoplastic endpoint was epidermal hyperplasia. In male mice, the BMD calculations failed when using the entire epidermal hyperplasia data set. When the calculations were repeated without the 2.7 mg/kg/day group, the BMDL₁₀ ranged from 1.6 to 2.8 mg/kg/day; when the 5.8 mg/kg/day group was eliminated, the comparable values were 0.88–0.93 mg/kg/day. In female mice, the BMDL₁₀ for epidermal hyperplasia ranged from 1.5 to 3.0 mg/kg/day. Higher values were obtained for epidermal suppurative inflammation (3.1–7.6 mg/kg/day) and epidermal ulceration (6.6–9.7 mg/kg/day) in male mice.

Table 8. Benchmark Dose Modeling Results for Select Neoplasms and Nonneoplastic Lesions in Male and Female Mice in the Two-year Dermal Study of Triclosan

Neoplasm or Nonneoplastic Lesion	BMDL ₁₀ (mg/kg/day)
Male	
Neoplasm	
Hepatocellular carcinoma	1.1–2.4
Nonneoplastic Lesions	
Epidermis, hyperplasia ^a	1.6–2.8
Epidermis, hyperplasia ^b	0.88–0.93
Epidermis, inflammation, suppurative	3.1–7.6
Epidermis, ulcer	6.6–9.7
Female	
Neoplasm	
Pancreas, islets, adenoma	11.7–12.3
Nonneoplastic Lesion	
Epidermis, hyperplasia	1.5–3.0

BMDL₁₀ = 95% lower bound on the benchmark dose corresponding to a 10% extra risk of the specified adverse effect over that observed in the vehicle control group. Benchmark calculations were conducted using gamma, logistic, log-logistic, log-probit, multistage, probit, and Weibull models to fit the incidences of neoplasms and nonneoplastic lesions and the doses of triclosan administered dermally. The values shown are the range of BMDLs calculated for a specific neoplasm or nonneoplastic lesion across the various models used.

^aCalculations were run using all dosed groups except the 2.7 mg/kg/day group.

^bCalculations were run using all dosed groups except the 5.8 mg/kg/day group.

Discussion

In the current 2-year mouse study, the survival of all dosed groups was similar to that of the vehicle control groups. There were significant decreases in body weight in the 12.5 mg triclosan/kg body weight/day (mg/kg/day) group for both sexes; nonetheless, the decrease was modest and did not exceed 8%. Minimal to mild gross lesions of the skin (dry flaking skin, scratches, thickened skin, and dark pigmentation) were observed in all dosed groups. The severity of these lesions was not sufficient to cause the removal of any animal from the study. There were no neoplasms observed at the site of triclosan application.

The dermal administration of triclosan resulted in increased incidences of hepatocellular neoplasms when compared to the vehicle control group, which is the most appropriate comparison. A positive trend in the incidence of hepatocellular carcinoma was noted in male mice, with the increase becoming significant at ≥ 5.8 mg/kg/day. The incidence of hepatocellular carcinoma in the vehicle control group was within the historical control range (10%–42%, average 25.16% for all routes of exposure), and the incidence in the 5.8 and 12.5 mg/kg/day groups was outside the historical control range. There was also a significant increase in the incidence of hepatocellular adenoma at 2.7 mg/kg/day. The incidence of hepatocellular adenoma or carcinoma (combined) was significantly increased in the ≥ 2.7 mg/kg/day groups, with a positive trend. Together these data indicate carcinogenic activity from the exposure to triclosan. The unusually high incidences of hepatocellular carcinoma and hepatocellular adenoma or carcinoma (combined) in the untreated control group (56% and 85%, respectively), compared to the vehicle control group (31% and 65%, respectively), and a lack of exposure-related nonneoplastic lesions in the liver, reduced confidence in the strength of the evidence. The incidence of hepatocellular carcinoma in the vehicle control males was within the historical control range, and the cause of the high incidence in the untreated control group, which was outside the historical control range, is unknown. Taken together, the significantly increased incidence of hepatocellular adenoma or carcinoma (combined) in male mice, relative to the vehicle control group, was *some evidence* of carcinogenic activity. This response did not occur in female mice.

The hepatocellular carcinoma incidence in untreated control male mice in the current study exceeded the NTP 5-year historical control incidence for all routes of exposure but is within the historical control range for dermal studies of Taconic B6C3F1 mice fed the NTP-2000 diet conducted between 1996 and 2010. The reason for the unusually high hepatocellular carcinoma incidence in the untreated control males in the current study is not known. However, the untreated control animals were not subjected to the same handling and dosing as the other groups, and this result does not negate the fact that there was a dose-related hepatocellular response in male mice administered triclosan compared to the vehicle control group.

Hepatocellular neoplasms have been reported in male and female CD-1 mice administered triclosan at dietary concentrations equivalent to doses of ≥ 30 mg/kg/day (summarized in Rodricks et al.³). Benchmark dose (BMD) modeling of hepatocellular carcinoma in the male B6C3F1/N mice indicated that the benchmark dose lower confidence limit (BMDL₁₀) ranged from 1.1 to 2.4 mg/kg/day, which was similar to that of epidermal hyperplasia.

The mechanism for the hepatic neoplasm induction in the current study is not known; however, evidence has been presented for a mode of action involving peroxisome proliferator-activated receptor alpha (PPAR α) and/or the constitutive androstane receptor (CAR).^{3; 28; 29} Other studies have shown that triclosan activates mouse, but not human, PPAR α ³⁰ and that triclosan induces hepatocyte proliferation in wild type C57BL/6 mice but not in mice transfected with human PPAR α .³¹

Triclosan has been shown to accelerate the development of hepatocellular carcinoma in male C57BL/6 *Car*^{+/-} and C57BL/6 *Car*^{-/-} mice initiated with *N,N*-diethylnitrosamine.³² This response was attributed to triclosan enhancing liver fibrosis and proliferation. Ethanol is metabolized to acetaldehyde, which reacts with DNA to result in a Schiff base that can be reduced to *N*²-ethyldeoxyguanosine.^{33; 34} The possibility exists that exposure to ethanol may result in tumor initiation through the formation of *N*²-ethyldeoxyguanosine, a DNA adduct that is known to miscode,³⁵ and the coadministration of triclosan could serve as a tumor promoter. Although the incidence of hepatic neoplasms was lower in the vehicle control group than in the untreated control group in the current study, that lower incidence is of unknown origin. In addition to hepatocellular carcinoma in male B6C3F1/N mice, triclosan induced a positive trend in pancreatic islet adenoma in female B6C3F1/N mice. Pancreatic islet adenoma is a rare neoplasm in female B6C3F1/N mice. In NTP 2-year studies conducted using Taconic B6C3F1/N mice and the NTP-2000 diet, control female mice have a mean incidence of 0.38% (range: 0%–2%) for all routes of exposure. Although there was a positive trend in the incidence of pancreatic islet adenoma, the occurrence of pancreatic islet adenomas and carcinomas in the control groups, along with the lack of exposure-related preneoplastic lesions, lowered confidence in the strength of the evidence. This was considered to be *equivocal evidence* of carcinogenic activity.

Neoplasms were not detected at the site of triclosan application in either sex. Histopathological examination of the site of triclosan application indicated the presence of epidermal hyperplasia in both sexes and epidermal suppurative inflammation and ulceration in male mice. Male mice had a significant increase in the incidences of epidermal hyperplasia at doses ≥ 2.7 mg/kg/day; in female mice, the increase became significant at 1.25 mg/kg/day. The increases in the incidences of epidermal suppurative inflammation and ulceration became significant at 12.5 mg/kg/day in male mice. BMD modeling of the skin lesions indicated that the BMDL₁₀ was 0.88–2.8 mg/kg/day for epidermal hyperplasia in male mice and 1.5–3.0 mg/kg/day in female mice. These BMDL₁₀ values are lower than those obtained in a previous 13-week dermal study.⁴ They are also lower than the BMDL₁₀ of 47 mg/kg/day, determined from kidney toxicity in hamsters, which was used to calculate margins of safety for triclosan.³ Likewise, they are also lower than the 12 mg/kg/day no-observed-adverse-effect level (NOAEL), determined from hepatotoxicity and decreased spleen weights of female rats, which was used by the Scientific Committee on Consumer Products³⁶ and Lee et al.³⁷ in their calculations of margins of safety for exposure to triclosan, or a NOAEL of 75 mg/kg/day determined from lifetime studies in hamsters, which was used to calculate the margin of safety following dermal administration of triclosan.³⁸

Male B6C3F1 mice receiving a single dermal administration of 10 mg/kg triclosan had a maximum plasma concentration (C_{\max}) of 26 μM , and mice receiving 100 mg/kg triclosan had a C_{\max} of 188 μM .⁵ These data suggest that mice receiving 1 mg/kg triclosan, a value similar to the BMDL₁₀ values for epidermal hyperplasia, would have a C_{\max} of approximately 2 μM . Humans exposed to a single oral dose of 4 mg triclosan had plasma concentrations of approximately 1 μM .³⁹ Likewise, oral exposure to 0.7 mg triclosan resulted in plasma concentrations of

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approximately $0.3 \mu\text{M}^{40}$; thus, dermal exposure equivalent to the BMDL_{10} values for epidermal hyperplasia in mice is expected to result in plasma concentrations that could occur in people who use mouthwash products containing triclosan.

Conclusions

Under the conditions of this 2-year dermal study, there was *some evidence of carcinogenic activity* of triclosan in male B6C3F1/N mice based on the increased incidences of hepatocellular adenoma or carcinoma (combined). There was *equivocal evidence of carcinogenic activity* of triclosan in female B6C3F1/N mice based on higher occurrences of pancreatic islet adenomas.

Dermal administration of triclosan resulted in increased incidences of nonneoplastic epidermal lesions at the site of application in male and female mice.

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Appendix A. Chemical Characterization and Dose Formulation Studies

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A.1. Procurement and Characterization

A.1.1. Triclosan

Triclosan was obtained from Alfa Aesar (Ward Hill, MA) in a single lot (5001A29X). Identity, purity, and stability analyses were conducted by the study laboratory at the U.S. Food and Drug Administration's National Center for Toxicological Research (NCTR, Jefferson, AR). Reports on analyses performed in support of the triclosan studies are on file at the National Institute of Environmental Health Sciences.

The identity of the test lot was evaluated using ^1H nuclear magnetic resonance (NMR) spectroscopy. The ^1H NMR spectrum was consistent with the structure of triclosan and identical to that of the reference standard (lot 1412854V, Sigma Aldrich, St. Louis, MO) (Figure A-1). No resonances due to contaminants were present in the spectrum except those produced by water and solvent. The identity of lot 5001A29X was further confirmed using mass spectrometry (MS) after separation by high-performance liquid chromatography (HPLC) with diode array detection (PDA) (Table A-1, System A). The MS spectrum was consistent with the structure of triclosan, which confirmed that the test lot corresponded to triclosan.

Purity evaluation was conducted by HPLC/PDA (Table A-1, System B) using a 1.0 mg/mL sample of the test lot dissolved in methanol. No impurities were identified using HPLC/PDA. The overall purity of lot 5001A29X was estimated to be 99.9% by comparing the peak area with the reference standard, which was consistent with the 99.0% purity indicated by the manufacturer. The ^1H NMR analysis of the test article was consistent with this purity determination. Additionally, the test lot matched the reference standard's HPLC retention time and ultraviolet (UV) spectrum. Repeated purity evaluation conducted at the end of the study confirmed a purity of 99.9%.

The test article was received in amber jars and stored at room temperature.

A.1.2. Ethanol

The 95% ethanol dose vehicle was obtained from Decon Laboratories (King of Prussia, PA) in a single lot (2801G). The identity of ethanol was confirmed using ^1H NMR spectroscopy. The NMR spectrum was consistent with the structure of ethanol, and there was no evidence of contamination by other proton-containing compounds (Figure A-2).

Purity evaluation of lot 2801G was conducted by the study laboratory using HPLC/PDA (Table A-1, System B). The chromatograms did not show any UV chromophore-containing components above background, confirming a purity consistent with the 95% purity specified by the vendor. Batches of the ethanol dose vehicle were evaluated for the presence of triclosan before and after the study, and no triclosan was detected.

The ethanol dose vehicle was received in amber glass containers and stored at room temperature.

A.2. Preparation and Analysis of Dose Formulations

Dose formulations of triclosan (lot 5001A29X) in 95% ethanol were prepared by Priority One Services (Alexandria, VA) following the protocols outlined in Table A-2. Dose formulations

were prepared at 1.25, 2.7, 5.8, and 12.5 mg/L (25 preparations; September 2013–August 2015) and were refrigerated, protected from light, and used within approximately 1 month of mixing.

Stability studies of the dose formulations stored at room (25°C) or refrigerated (2°C–8°C) temperatures were conducted using HPLC/PDA (Table A-1, System B). The lowest (1.25 mg/mL) and highest (12.5 mg/mL) dose formulations were evaluated after 8 weeks at refrigerated temperatures or 4 weeks at room temperature. Stability for up to 56 days under refrigerated temperatures was confirmed. The dose formulations were also found to be homogeneous, as the percent coefficient of variation (%CV) values of three replicate samples per dose preparation were all $\leq 10\%$, with one exception: the 2.7 mg/mL formulation prepared on January 13, 2014, had a %CV value of 10.6%.

Analyses of preadministration dose formulations were conducted by the study laboratory using HPLC/PDA (Table A-1, System B). All dose formulations were within 10% of the target concentrations, with one exception: the 12.5 mg/mL formulation prepared on February 19, 2014, had a concentration of only 1.31 mg/mL. However, this dose was remixed on February 21, 2014, and the new solution was within 10% of the target concentration (Table A-3).

A.3. Analysis of Triclosan in Experimental Background Materials

The animal feed (NTP-2000 Irradiated Rodent Diet) and drinking water (Millipore-filtered drinking water) were analyzed for the presence of background triclosan using ultra-performance liquid chromatography (UPLC) with MS (Table A-1, System C). Animal feed samples were ground to a powder and extracted with acetonitrile. The acetonitrile supernatant was assayed by UPLC-MS and compared with feed samples spiked at 1 ppm triclosan. Although a prominent triclosan peak was observed in the spiked samples, the peak was not detectable in the unspiked samples, indicating that any possible background levels of triclosan in the feed were not present at or above 1 ppm. Additionally, peak areas observed for triclosan in the three drinking water samples were negligible in comparison with water samples spiked at 1 ppm triclosan, indicating that the drinking water triclosan levels were not present at or above 1 ppm.

Table A-1. Liquid Chromatography Systems Used in the Two-year Dermal Study of Triclosan

Chromatography	Detection System	Column	Mobile Phase
System A			
High-performance liquid chromatography	Diode array detector (200–400 nm) and tandem mass spectrometry (ESI)	Waters Acquity BEH C18 (2.1 × 50 mm, 1.7 μm particle size)	A: Acetonitrile B: Water Gradient program: A:B 20:80 to 90:10 in 4 min 500 μL/min flow rate

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Chromatography	Detection System	Column	Mobile Phase
System B			
High-performance liquid chromatography	Diode array detector (190–400 nm)	Waters Novapak-C18 (3.9 mm × 150 mm, 4 μm particle size)	A: 5 mM phosphate buffer:methanol, pH 2.5 (95:5) B: 5 mM phosphate buffer:methanol, pH 2.5 (5:95) Gradient program: A:B 100:0 hold for 3 min; 0:100 for 9.5 min at gradient curve 6; 0:100 for 0.75 min at gradient curve 11; 100:0 for 26.75 min at gradient curve 11.
System C			
Ultra-performance liquid chromatography	Mass spectrometry (ESI)	Waters Acquity BEH C18 (1.2 mm × 50 mm, 1.7 μm particle size)	A: Water B: Acetonitrile Gradient program: A:B 80:20 hold for 4 min; 10:90 for 0.1 min at gradient curve 6; 80:20 for 1.9 min at gradient curve 6.

ESI = electrospray ionization.

Table A-2. Preparation and Storage of Dose Formulations Administered to Mice in the Two-year Dermal Study of Triclosan

Preparation
Formulations were prepared by weighing the required amount of triclosan powder into a glass beaker and pouring ~70% of the required ethanol volume into the beaker. Formulations were stirred with a stir bar for ~5 minutes and transferred to a volumetric flask. The remaining ethanol was added with a syringe or pipette, and the flask was inverted several times to mix. The formulations were transferred into the required number of amber glass, screw-top bottles for dosing. Additional mixing occurred as needed during storage.
Chemical Lot Number
5001A29X (Alfa Aesar, Ward Hill, MA)
Vehicle Lot Number
2801G (Decon Laboratories, King of Prussia, PA)
Maximum Storage Time
56 days
Storage Conditions
Amber glass bottles at 2°C–8°C
Study Laboratory
National Center for Toxicological Research (Jefferson, AR)

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Table A-3. Results of Analyses of Dose Formulations Administered to Mice in the Two-year Dermal Study of Triclosan

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL) ^a	Difference from Target (%)
September 5, 2013	September 10, 2013	0	BLOQ	NA
		1.25	1.29 ± 0.09	3.2
		2.7	2.93 ± 0.22	8.5
		5.8	6.03 ± 0.17	4.0
		12.5	12.1 ± 0.18	-3.2
September 23, 2013	September 25, 2013	0	BLOQ	NA
		1.25	1.20 ± 0.05	-4.0
		2.7	2.68 ± 0.2	-0.7
		5.8	5.86 ± 0.2	1.0
		12.5	13.1 ± 1.0	4.8
September 30, 2013	October 9, 2013	0	BLOQ	NA
		1.25	1.24 ± 0.10	-0.8
		2.7	2.80 ± 0.01	3.7
		5.8	5.70 ± 0.46	-1.7
		12.5	13.6 ± 0.4	8.8
November 4, 2013	November 6, 2013	0	BLOQ	NA
		1.25	1.32 ± 0.01	5.6
		2.7	2.85 ± 0.01	5.6
		5.8	5.83 ± 0.07	0.5
		12.5	11.9 ± 1.2	-4.8
December 10, 2013	December 16, 2013	0	BLOQ	NA
		1.25	1.16 ± 0.09	-7.2
		2.7	2.51 ± 0.25	-7.0
		5.8	5.68 ± 0.4	-2.1
		12.5	12.6 ± 0.16	0.8
January 13, 2014	January 15, 2014	0	BLOQ	NA
		1.25	1.25 ± 0.11	0.0
		2.7	2.96 ± 0.31	9.6
		5.8	6.04 ± 0.48	4.1
		12.5	13.0 ± 1.25	4.0
February 19, 2014	February 21, 2014	0	BLOQ	NA
		1.25	1.37 ± 0.06	9.6
		2.7	2.72 ± 0.21	0.7

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Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL) ^a	Difference from Target (%)
March 18, 2014	March 20, 2014	5.8	5.99 ± 0.22	3.3
		12.5 ^b	12.0 ± 0.35	-4.0
		0	BLOQ	NA
		1.25	1.37 ± 0.05	9.6
		2.7	2.74 ± 0.19	1.5
April 21, 2014	April 22, 2014	5.8	5.75 ± 0.31	-0.9
		12.5	13.1 ± 0.54	4.8
		0	BLOQ	NA
		1.25	1.25 ± 0.09	0.0
		2.7	2.72 ± 0.01	0.7
May 21, 2014	May 23, 2014	5.8	5.69 ± 0.11	-1.9
		12.5	12.9 ± 0.2	3.2
		0	BLOQ	NA
		1.25	1.29 ± 0.01	3.2
		2.7	2.75 ± 0.02	1.9
June 18, 2014	June 19, 2014	5.8	5.95 ± 0.05	2.6
		12.5	12.8 ± 0.10	2.4
		0	BLOQ	NA
		1.25	1.26 ± 0.05	0.8
		2.7	2.77 ± 0.01	2.6
July 15, 2014	July 16, 2014	5.8	5.9 ± 0.02	1.7
		12.5	12.6 ± 0.07	0.8
		0	BLOQ	NA
		1.25	1.25 ± 0.01	0.0
		2.7	2.69 ± 0.03	-0.4
August 19, 2014	August 21, 2014	5.8	5.76 ± 0.04	-0.7
		12.5	12.3 ± 0.33	-1.6
		0	BLOQ	NA
		1.25	1.23 ± 0.02	-1.6
		2.7	2.61 ± 0.03	-3.3
September 22, 2014	September 25, 2014	5.8	5.49 ± 0.07	-5.3
		12.5	12.0 ± 0.57	-4.0
		0	BLOQ	NA
		1.25	1.31 ± 0.06	4.8
		2.7	2.78 ± 0.1	3.0

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Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL) ^a	Difference from Target (%)
		5.8	5.91 ± 0.57	1.9
		12.5	13.4 ± 0.13	7.2
October 21, 2014	October 24, 2014	0	BLOQ	NA
		1.25	1.36 ± 0.00	8.8
		2.7	2.75 ± 0.19	1.9
		5.8	6.18 ± 0.01	6.6
		12.5	13.3 ± 0.36	6.4
November 17, 2014	November 20, 2014	0	BLOQ	NA
		1.25	1.26 ± 0.02	0.8
		2.7	2.65 ± 0.02	-1.9
		5.8	5.45 ± 0.12	-6.0
		12.5	12.0 ± 0.12	-4.0
December 15, 2014	December 19, 2014	0	BLOQ	NA
		1.25	1.24 ± 0.04	-0.8
		2.7	2.56 ± 0.07	-5.2
		5.8	5.64 ± 0.21	-2.8
		12.5	11.6 ± 0.34	-7.2
January 13, 2015	January 16, 2015	0	BLOQ	NA
		1.25	1.22 ± 0.04	-2.4
		2.7	2.64 ± 0.04	-2.2
		5.8	6.18 ± 0.05	6.6
		12.5	12.6 ± 0.9	0.8
February 18, 2015	February 27, 2015	0	BLOQ	NA
		1.25	1.24 ± 0.09	-0.8
		2.7	2.64 ± 0.12	-2.2
		5.8	5.71 ± 0.17	-1.6
		12.5	12.4 ± 0.4	-0.8
March 16, 2015	March 20, 2015	0	BLOQ	NA
		1.25	1.33 ± 0.04	6.4
		2.7	2.63 ± 0.2	-2.6
		5.8	6.07 ± 0.38	4.7
		12.5	12.8 ± 0.6	2.4
April 14, 2015	April 16, 2015	0	BLOQ	NA
		1.25	1.33 ± 0.1	6.4
		2.7	2.79 ± 0.1	3.3

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Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL) ^a	Difference from Target (%)
May 18, 2015	May 20, 2015	5.8	5.86 ± 0.27	1.0
		12.5	13.2 ± 0.1	5.6
		0	BLOQ	NA
		1.25	1.24 ± 0.01	-0.8
		2.7	2.7 ± 0.04	0.0
June 15, 2015	June 17, 2015	5.8	5.84 ± 0.06	0.7
		12.5	12.5 ± 0.05	0.0
		0	BLOQ	NA
		1.25	1.27 ± 0.02	1.6
		2.7	2.7 ± 0.07	0.0
July 20, 2015	July 24, 2015	5.8	5.71 ± 0.04	-1.6
		12.5	12.1 ± 0.4	-3.2
		0	BLOQ	NA
		1.25	1.20 ± 0.01	-4.0
		2.7	2.54 ± 0.02	-5.9
August 24, 2015	August 27, 2015	5.8	5.58 ± 0.04	-3.8
		12.5	12.0 ± 0.3	-4.0
		0	BLOQ	NA
		1.25	1.19 ± 0.01	-4.8
		2.7	2.61 ± 0.04	-3.3
		5.8	5.62 ± 0.08	-3.1
		12.5	12.3 ± 0.2	-1.6

BLOQ = below the limit of quantification; NA = not applicable.

^aData are presented as mean ± standard deviation.

^bPrepared on February 21, 2014.

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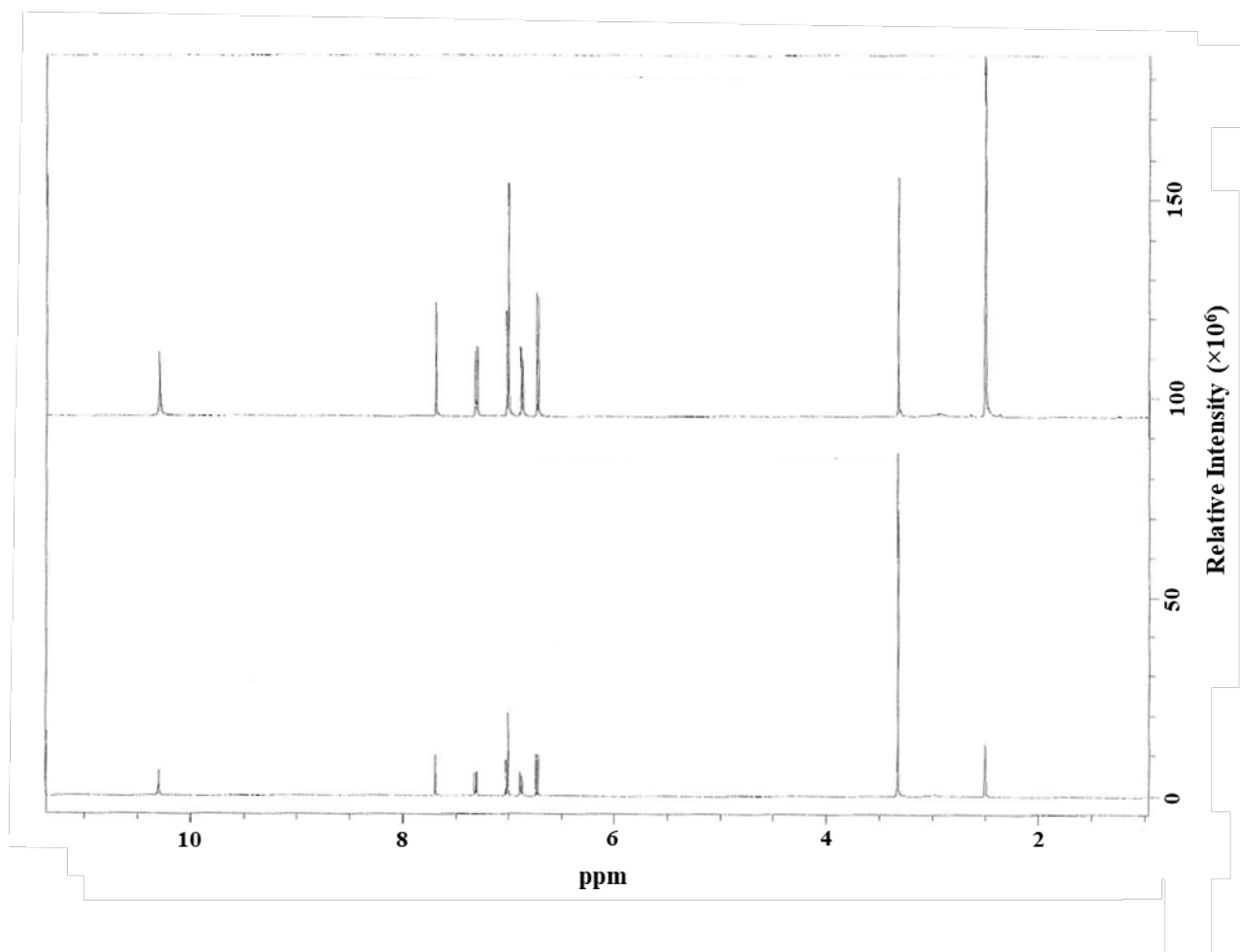


Figure A-1. ¹H Nuclear Magnetic Resonance Spectrum of Triclosan

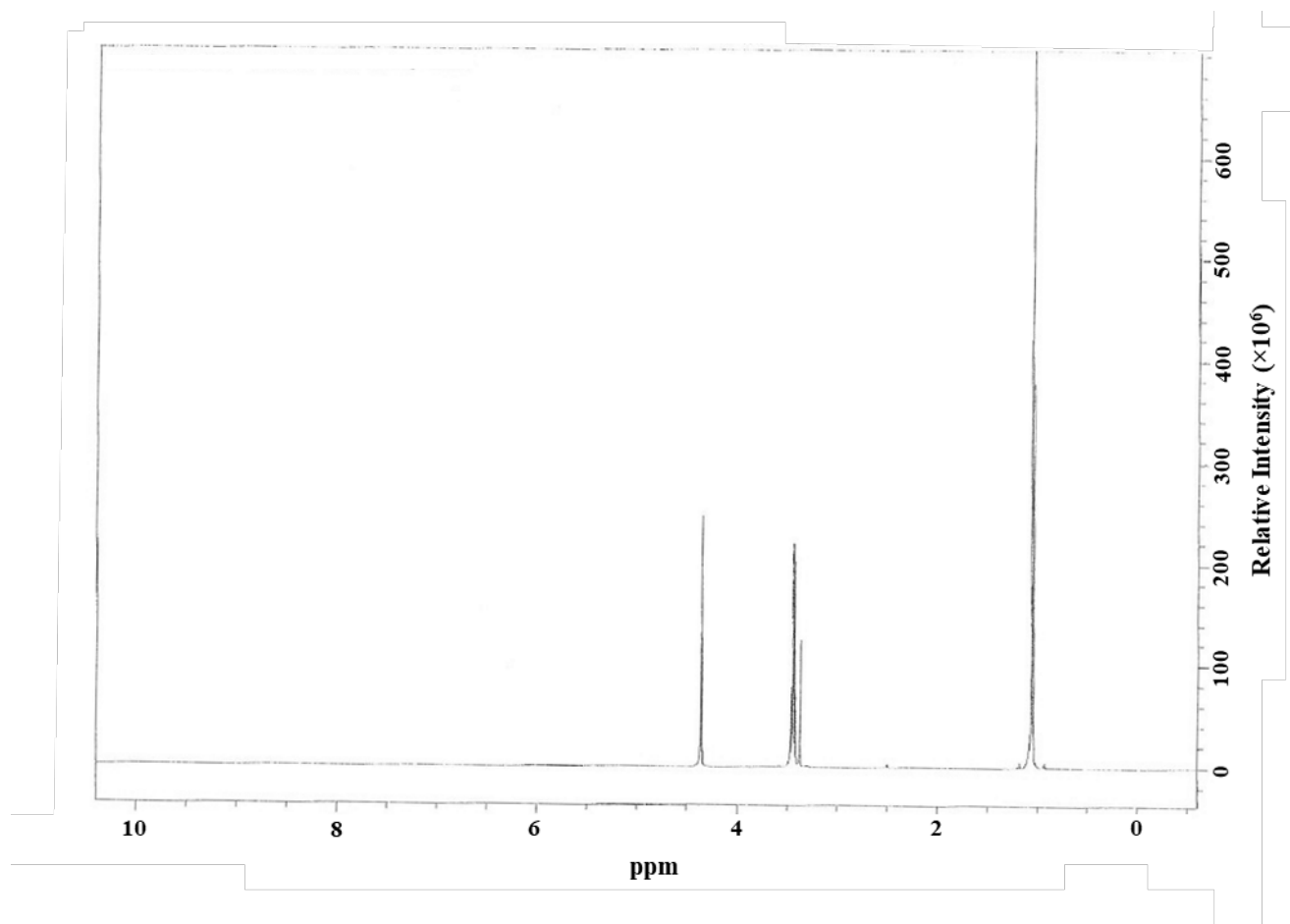


Figure A-2. ¹H Nuclear Magnetic Resonance Spectrum of Ethanol

Appendix B. Ingredients, Nutrient Composition, and Contaminant Levels in NTP-2000 Mouse Ration

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B.1. NTP-2000 Feed**Table B-1. Ingredients of NTP-2000 Mouse Ration**

Ingredients	Percent by Weight
Ground Hard Winter Wheat	23.00
Ground #2 Yellow Shelled Corn	22.44
Wheat Middlings	15.00
Oat Hulls	8.50
Alfalfa Meal (Dehydrated, 17% Protein)	7.50
Purified Cellulose	5.50
Soy Protein Concentrate	4.00
Fish Meal (60% Protein)	4.00
Corn Oil (Without Preservatives)	3.00
Soy Oil (Without Preservatives)	3.00
Dried Brewer's Yeast	1.00
Calcium Carbonate (USP)	0.90
Vitamin Premix ^a	0.50
Mineral Premix ^b	0.50
Calcium Phosphate, Dibasic (USP)	0.40
Sodium Chloride	0.30
Choline Chloride (70% Choline)	0.26
Methionine	0.20

USP = United States Pharmacopeia.

^aWheat middlings as carrier.

^bCalcium carbonate as carrier.

Table B-2. Vitamins and Minerals in NTP-2000 Mouse Ration

	Amount ^a	Source
Vitamins		
Vitamin A	4,000 IU	Stabilized vitamin A palmitate or acetate
Vitamin D	1,000 IU	D-activated animal sterol
Vitamin K	1.0 mg	Menadione sodium bisulfite complex
α -Tocopheryl Acetate	100 IU	–
Niacin	23 mg	–
Folic Acid	1.1 mg	–
d-Pantothenic Acid	10 mg	d-Calcium pantothenate
Riboflavin	3.3 mg	–
Thiamine	4 mg	Thiamine mononitrate
B12	52 μ g	–

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	Amount ^a	Source
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	d-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

^aPer kg of finished diet.

Table B-3. Nutrient Composition of NTP-2000 Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by Weight)	14.72 ± 0.4622	14.1–15.5	13
Crude Fat (% by Weight)	7.96 ± 0.9014	5.1–8.7	13
Crude Fiber (% by Weight)	9.22 ± 0.6446	7.7–10.1	13
Ash (% by Weight)	5.33 ± 1.320	4.72–9.7	13
Amino Acids (% of Total Diet)			
Arginine	0.806 ± 0.074	0.67–0.97	30
Cystine	0.220 ± 0.0212	0.15–0.25	30
Glycine	0.702 ± 0.037	0.62–0.8	30
Histidine	0.341 ± 0.0692	0.27–0.68	30
Isoleucine	0.548 ± 0.0393	0.43–0.66	30
Leucine	1.096 ± 0.062	0.96–1.24	30
Lysine	0.699 ± 0.1027	0.31–0.86	30
Methionine	0.409 ± 0.041	0.26–0.49	30
Phenylalanine	0.623 ± 0.046	0.471–0.72	30
Threonine	0.513 ± 0.041	0.43–0.61	30
Tryptophan	0.156 ± 0.0262	0.11–0.2	30
Tyrosine	0.423 ± 0.649	0.28–0.54	30
Valine	0.666 ± 0.039	0.55–0.73	30

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Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Essential Fatty Acids (% of Total Diet)			
Linoleic	3.94 ± 0.233	3.49–4.55	30
Linolenic	0.31 ± 0.030	0.21–0.368	30
Vitamins			
Vitamin A (IU/kg)	4,225 ± 83.87	3,040–6,210	13
Vitamin D (IU/kg)	1,000 ^a	–	–
α-Tocopherol (ppm)	2,376 ± 12,602	13.6–69,100	30
Thiamine (ppm) ^b	13.6 ± 20.31	6.6–81.0	13
Riboflavin (ppm)	8.17 ± 2.792	4.2–17.5	30
Niacin (ppm)	79.19 ± 8.50	66.4–98.2	30
Pantothenic Acid (ppm)	26.33 ± 10.87	17.4–81	30
Pyridoxine (ppm) ^b	9.72 ± 2.018	6.44–14.3	30
Folic Acid (ppm)	1.6 ± 0.44	1.15–3.27	30
Biotin (ppm)	0.33 ± 0.10	0.2–0.704	30
Vitamin B ₁₂ (ppb)	50.06 ± 34.34	18.3–174	30
Choline (as Chloride) (ppm)	2,572 ± 634	1,160–3,790	30
Minerals			
Calcium (%)	0.950 ± 0.0532	0.863–1.02	13
Phosphorus (%)	0.582 ± 0.032	0.525–0.639	13
Potassium (%)	0.680 ± 0.029	0.626–0.733	30
Chloride (%)	0.391 ± 0.044	0.3–0.517	30
Sodium (%)	0.194 ± 0.0274	0.153–0.283	30
Magnesium (%)	0.217 ± 0.053	0.185–0.49	30
Iron (ppm)	190.43 ± 36.106	135–311	30
Manganese (ppm)	50.02 ± 9.27	21.0–73.1	30
Zinc (ppm)	56.81 ± 25.25	42.5–184.0	30
Copper (ppm)	7.61 ± 2.46	3.21–16.3	30
Iodine (ppm)	0.514 ± 0.217	0.0–0.972	30
Chromium (ppm)	1.119 ± 1.16	0.33–3.97	30
Cobalt (ppm)	0.219 ± 0.150	0.0857–0.864	28

^aFrom formulation.

^bAs hydrochloride.

Table B-4. Contaminant Levels in NTP-2000 Mouse Ration

	Mean ± Standard Deviation	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.2228 ± 0.018	0.196–0.262	13
Cadmium (ppm)	0.0507 ± 0.004	0.045–0.058	13
Lead (ppm)	0.1434 ± 0.1458	0.06–0.621	13
Mercury (ppm)	0.0104 ± 0.0014	0.01–0.015	13
Selenium (ppm)	0.1871 ± 0.0318	0.135–0.251	13
Aflatoxins (ppb) ^a	5	–	13
Nitrate Nitrogen (ppm) ^b	15.692 ± 5.813	9.91–27.4	13
Nitrite Nitrogen (ppm) ^b	0.395 ± 0.245	0.12–0.63	13
BHA (ppm) ^c	1.412 ± 1.064	1.0–4.76	13
BHT (ppm) ^{a,c}	1	–	13
Aerobic Plate Count (CFU/g)	10.08 ± 3.883	1.0–20.0	13
Coliform (MPN/g) ^a	3	–	13
<i>Escherichia coli</i> (MPN/g)	4.615 ± 3.070	3.0–10.0	13
<i>Salmonella</i> (MPN/g)	Negative	–	13
Total Nitrosamines (ppb) ^d	9.964 ± 4.071	0.0–14.1	11
N-Nitrosodimethylamine (ppb) ^d	2.51 ± 2.365	0.0–7.9	11
N-Nitrosopyrrolidine (ppb) ^d	7.455 ± 3.02	0.0–11.9	11
Pesticides (ppm)			
α-BHC ^a	<0.01	–	13
β-BHC ^a	<0.02	–	13
γ-BHC ^a	<0.01	–	13
δ-BHC ^a	<0.01	–	13
Heptachlor ^a	<0.01	–	13
Aldrin ^a	<0.01	–	13
Heptachlor Epoxide ^a	<0.01	–	13
DDE ^a	<0.01	–	13
DDD ^a	<0.01	–	13
DDT ^a	<0.01	–	13
HCB ^a	<0.01	–	13
Mirex ^a	<0.01	–	13
Methoxychlor ^a	<0.05	–	13
Dieldrin ^a	<0.01	–	13
Endrin ^a	<0.01	–	13

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	Mean ± Standard Deviation	Range	Number of Samples
Telodrin ^a	<0.01	–	13
Chlordane ^a	<0.05	–	13
Toxaphene ^a	<0.10	–	13
Estimated PCBs ^a	<0.20	–	13
Ronnel ^a	<0.01	–	13
Ethion ^a	<0.02	–	13
Trithion ^a	<0.05	–	13
Diazinon ^a	<0.10	–	13
Methyl Chlorpyrifos	0.139 ± 0.1076	0.02–0.686	13
Methyl Parathion ^a	<0.02	–	13
Ethyl Parathion ^a	<0.02	–	13
Malathion	0.107 ± 0.155	0.02–0.585	13
Endosulfan I ^a	<0.01	–	13
Endosulfan II ^a	<0.01	–	13
Endosulfan Sulfate ^a	<0.03	–	13

All samples were irradiated.

BHA = butylated hydroxyanisole; BHT = butylated hydroxytoluene; CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride; DDE = dichlorodiphenyldichloroethylene; DDD = dichlorodiphenyldichloroethane; DDT = dichlorodiphenyltrichloroethane; HCB = hexachlorobenzene; PCB = polychlorinated biphenyl.

^aAll values were below the detection limit. The detection limit is given as the mean.

^bSources of contamination include alfalfa, grains, and fish meal.

^cSources of contamination include soy oil and fish meal.

^dAll values were corrected for percent recovery.

Appendix C. Sentinel Animal Program

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Table C-1. Methods and Results for Sentinel Animal Testing in Male and Female Mice	C-3
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C.1. Methods

Rodents used in the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that might affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicological evaluation of test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) or exposed animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals come from the same production source and weanling groups as the animals used for the studies of test compounds.

For this toxicology and carcinogenesis study, blood samples were collected from each sentinel animal and allowed to clot, and the serum was separated. All samples were processed appropriately with serology by IDEXX BioResearch (formerly Rodent Animal Diagnostic Laboratory [RADIL], University of Missouri, Columbia, MO) for determination of the presence of pathogens. Evaluation for endo- and ectoparasites was performed at the U.S. Food and Drug Administration's National Center for Toxicological Research.

The laboratory methods and agents for which testing was performed in the sentinel animals are tabulated in Table C-1 below; the times at which samples were collected during the study are also listed.

C.2. Results

All test results were negative.

Table C-1. Methods and Results for Sentinel Animal Testing in Male and Female Mice

Collection Time Points	Quarantine	3 Months	6 Months	9 Months	12 Months	15 Months	18 Months	21 Months	Study Termination
Number Examined (Males/Females)	12/12	2/2	2/2	2/2	2/2	2/2	2/2	2/2	2/2
Method/Test									
Multiplex Fluorescent Immunoassay (MFI)									
Ectromelia	–	–	–	–	–	–	–	–	–
Epizootic diarrhea of infant mice (EDIM)	–	–	–	–	–	–	–	–	–
Lymphocytic choriomeningitis virus (LCM)	–	–	–	–	–	–	–	–	–
<i>Mycoplasma pulmonis</i>	–	–	–	–	–	–	–	–	–
Mouse hepatitis virus (MHV)	–	–	–	–	–	–	–	–	–
Parvo NS-1	–	–	–	–	–	–	–	–	–
Polyoma	–	–	–	–	–	–	–	–	–
Mouse parvovirus (MPV)	–	–	–	–	–	–	–	–	–
Minute virus of mice (MMV)	–	–	–	–	–	–	–	–	–
Pneumonia virus of mice (PVM)	–	–	–	–	–	–	–	–	–
Reovirus Type 3 (REO3)	–	–	–	–	–	–	–	–	–
Sendai	–	–	–	–	–	–	–	–	–
Theiler's murine encephalomyelitis virus (TMEV)	–	–	–	–	–	–	–	–	–
In-house Evaluation									
Endoparasite evaluation	–	–	–	–	–	–	–	–	–
Ectoparasite evaluation	–	–	–	–	–	–	–	–	–
Polymerase Chain Reaction (PCR)									
<i>Helicobacter hepaticus</i>	–	–	–	–	–	–	–	–	–
Routine Culturing									
<i>Bordetella bronchiseptica</i>	–	–	–	–	–	–	–	–	–
<i>Citrobacter freundii</i>	–	–	–	–	–	–	–	–	–
<i>Corynebacterium kutscheri</i>	–	–	–	–	–	–	–	–	–

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Collection Time Points	Quarantine	3 Months	6 Months	9 Months	12 Months	15 Months	18 Months	21 Months	Study Termination
<i>Erysipelothrix rhusiopathiae</i>	-	-	-	-	-	-	-	-	-
<i>Klebsiella oxytoca</i>	-	-	-	-	-	-	-	-	-
<i>Klebsiella pneumonia</i>	-	-	-	-	-	-	-	-	-
<i>Listeria monocytogenes</i>	-	-	-	-	-	-	-	-	-
<i>Pasteurella pneumotropica</i>	-	-	-	-	-	-	-	-	-
<i>Pasteurella multocida</i>	-	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-	-
<i>Salmonella</i> sp.	-	-	-	-	-	-	-	-	-
<i>Streptococcus pneumoniae</i>	-	-	-	-	-	-	-	-	-

- = negative.

Appendix D. Benchmark Dose Analysis

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D.1. Methods

Benchmark doses (BMD) and the lower 95% confidence limits (BMDL) were calculated using U.S. Environmental Protection Agency Benchmark Dose Software (BMDS) version 2.4.0.70 (<https://www.epa.gov/bmds>). The calculations were conducted using gamma, logistic, log-logistic, log-probit, multistage, probit, and Weibull models to fit the incidences of neoplasms and nonneoplastic lesions and the doses of triclosan administered dermally. The BMD₁₀ and BMDL₁₀ were defined as the dose that caused a 10% excess risk of the specified adverse effect over that observed in the vehicle control group and as the 95% lower bound on the BMD corresponding to a 10% extra risk, respectively.

D.2. Results

BMD modeling was conducted on neoplasms (hepatocellular carcinoma in male mice and pancreatic islet adenoma in female mice) and nonneoplastic lesions (hyperplasia, suppurative inflammation, and ulceration of the epidermis in male mice and hyperplasia of the epidermis, pancreatic ductal dilatation, and pituitary gland angiectasis and pars distalis hyperplasia in female mice) (Table D-1). The 95% lower bound on the BMD corresponding to a 10% extra risk (BMDL₁₀) for hepatocellular carcinoma in male mice ranged from 1.1 to 2.4 mg triclosan/kg body weight/day (mg/kg/day), whereas the BMDL₁₀ for pancreatic islet adenoma in female mice ranged from 11.7 to 12.3 mg/kg/day. In both male and female mice, the most sensitive nonneoplastic endpoint was epidermal hyperplasia. In male mice, the BMD calculations failed when using the entire epidermal hyperplasia data set. When the calculations were repeated without the 2.7 mg/kg/day group, the BMDL₁₀ ranged from 1.6 to 2.8 mg/kg/day; when the 5.8 mg/kg/day group was eliminated, the comparable values were 0.88–0.93 mg/kg/day. In female mice, the BMDL₁₀ for epidermal hyperplasia ranged from 1.5 to 3.0 mg/kg/day. Higher values were obtained for epidermal suppurative inflammation (3.1–7.6 mg/kg/day) and epidermal ulceration (6.6–9.7 mg/kg/day) in male mice and for pituitary gland angiectasis (5.1–8.3 mg/kg/day) and pars distalis hyperplasia (7.0–9.2 mg/kg/day) and pancreatic duct dilatation (11.2–11.6 mg/kg/day) in female mice.

Table D-1. Benchmark Dose Modeling Results for Select Neoplasms and Nonneoplastic Lesions in Male and Female Mice in the Two-year Dermal Study of Triclosan

Model	AIC	Fitted Model ^a	GOF	BMD ₁₀	BMDL ₁₀
Male					
Epidermis, Hyperplasia					
Gamma ^{b,c}	212.0	0.0009	0.0007	1.321	0.773
Logistic ^{b,c}	214.7	0.0003	0.0002	2.283	1.910
Log-logistic ^{b,c}	214.0	0.0003	0.0003	1.410	0.910
Log-probit ^{b,c}	213.3	0.0004	0.0004	1.464	0.991
Multistage ^{b,c}	211.1	0.0013	0.0011	1.198	0.807
Probit ^{b,c}	213.7	0.0005	0.0003	2.149	1.819
Weibull ^{b,c}	211.7	0.0010	0.0008	1.314	0.796

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Model	AIC	Fitted Model^a	GOF	BMD₁₀	BMDL₁₀
Epidermis, Hyperplasia (minus the 2.7 mg/kg/day group)					
Gamma	133.1	0.489	0.485	2.987	1.808
Logistic	131.2	0.755	0.774	3.171	2.551
Log-logistic	133.6	0.316	0.324	3.851	2.572
Log-probit	133.7	0.297	0.308	3.968	2.771
Multistage	133.2	0.467	0.467	2.672	1.551
Probit	130.9	0.897	0.903	2.928	2.365
Weibull	133.1	0.489	0.485	2.987	1.808
Epidermis, Hyperplasia (minus the 5.8 mg/kg/day group)					
Gamma ^c	140.5	0.025	0.030	1.100	0.642
Logistic ^{b,c}	150.1	0.0002	0.0003	2.015	1.602
Log-logistic	138.2	0.098	0.109	1.262	0.884
Log-probit	137.9	0.116	0.125	1.303	0.932
Multistage ^c	142.0	0.011	0.016	0.849	0.561
Probit ^{b,c}	149.4	0.0004	0.0004	1.962	1.608
Weibull ^c	141.0	0.018	0.024	1.001	0.602
Epidermis, Inflammation, Suppurative					
Gamma	110.0	0.931	0.900	6.160	4.236
Logistic	113.9	0.429	0.639	9.383	7.568
Log-logistic	109.9	0.941	0.920	6.020	4.005
Log-probit	111.9	0.857	0.853	5.708	3.105
Multistage	110.0	0.931	0.900	6.160	4.236
Probit	113.7	0.469	0.665	8.994	7.101
Weibull	110.0	0.931	0.900	6.160	4.236
Epidermis, Ulcer					
Gamma	81.2	0.540	0.508	12.884	6.865
Logistic	81.6	0.472	0.578	13.086	9.711
Log-logistic	81.2	0.544	0.503	12.845	6.697
Log-probit	80.5	0.696	0.699	17.731	6.553
Multistage	81.2	0.540	0.507	12.884	6.865
Probit	81.5	0.479	0.572	13.128	9.360
Weibull	81.2	0.540	0.507	12.884	6.865
Hepatocellular Carcinoma					
Gamma	324.3	0.836	0.836	2.636	1.591
Logistic	324.8	0.729	0.729	3.429	2.353
Log-logistic	324.0	0.912	0.912	2.081	1.101

Triclosan, NTP TR 604

Model	AIC	Fitted Model ^a	GOF	BMD ₁₀	BMDL ₁₀
Log-probit ^d	325.8	0.866	0.866	1.417	0.017
Multistage	324.3	0.836	0.836	2.636	1.591
Probit	324.8	0.733	0.732	3.401	2.335
Weibull	324.3	0.836	0.836	2.636	1.591
Female					
Epidermis, Hyperplasia					
Gamma ^c	183.6	0.006	0.030	4.824	1.366
Logistic	178.2	0.078	0.166	3.594	2.998
Log-logistic ^c	183.5	0.007	0.032	4.954	2.647
Log-probit ^{b,c}	186.7	0.002	0.002	1.928	1.361
Multistage	178.4	0.074	0.071	2.114	1.461
Probit	178.2	0.080	0.148	3.285	2.758
Weibull ^c	182.4	0.011	0.034	3.823	1.468
Pancreas, Duct Dilatation					
Gamma	39.0	0.425	0.389	13.520	11.446
Logistic	39.2	0.393	0.381	14.781	11.628
Log-logistic	39.2	0.382	0.487	20.488	11.195
Log-probit	39.3	0.370	0.496	24.011	11.232
Multistage	39.1	0.395	0.488	18.402	11.254
Probit	39.2	0.387	0.387	15.396	11.480
Weibull	39.0	0.425	0.389	12.905	11.424
Pancreas, Islets, Adenoma					
Gamma	39.0	0.425	0.389	13.520	11.752
Logistic	40.4	0.235	0.178	15.720	11.813
Log-logistic	39.0	0.425	0.389	12.922	11.833
Log-probit	41.0	0.248	0.221	13.190	11.675
Multistage	40.3	0.248	0.309	19.173	12.272
Probit	40.6	0.215	0.177	16.973	11.847
Weibull	39.0	0.425	0.389	12.905	11.859
Pituitary Gland, Angiectasis					
Gamma	111.3	0.580	0.593	12.137	5.991
Logistic	109.2	0.798	0.800	11.297	8.313
Log-logistic	111.3	0.580	0.592	12.154	5.811
Log-probit	111.3	0.580	0.593	12.106	5.100
Multistage	111.3	0.578	0.587	11.232	5.988
Probit	109.3	0.791	0.795	11.211	7.945

Triclosan, NTP TR 604

Model	AIC	Fitted Model^a	GOF	BMD₁₀	BMDL₁₀
Weibull	111.3	0.580	0.592	12.162	5.991
Pituitary Gland, Pars Distalis, Hyperplasia					
Gamma	143.9	0.357	0.421	11.924	9.142
Logistic	146.0	0.147	0.247	11.466	7.567
Log-logistic	143.9	0.358	0.422	12.263	9.139
Log-probit	145.9	0.199	0.245	12.106	9.068
Multistage	145.5	0.185	0.280	11.841	7.046
Probit	146.1	0.141	0.240	11.623	7.318
Weibull	143.9	0.358	0.422	12.277	9.169

AIC = Akaike information criterion; GOF = goodness of fit p value; BMD₁₀ = benchmark dose corresponding to a 10% extra risk of the specified adverse effect over that observed in the vehicle control group; BMDL₁₀ = 95% lower bound on the benchmark dose corresponding to a 10% extra risk of the specified adverse effect over that observed in the vehicle control group

^aP value of fitted model compared to the full model.

^bModel rejected because scaled residual for one or more of the doses in GOF determination was >|2.00|.

^cModel rejected because fitted model and/or GOF p value ≤0.05.

^dDue to the disparity between the BMDL₁₀ obtained from the log-probit model and the BMDL₁₀ obtained from the other models, the log-probit BMDL₁₀ value was not considered further.

Appendix E. Supplemental Data

Tables with supplemental data can be found here: <https://doi.org/10.22427/NTP-DATA-TR-604>.²⁷

E.1. Two-year Study – Mice

E.1.1. Data Tables

E01 – Animal Removal Summary by Treatment Group

0303902_E01_Animal_Removal_Summary_by_Treatment_Group.pdf

E02 – Animals Removed from Experiment

0303902_E02_Animals_Removed_from_Experiment.pdf

Female Growth Curves

0303902_Female_Growth_Curves.pdf

Female Survival and Body Weights

Female_Survival_and_Body_Weights_508c.pdf

Male Growth Curves

0303902_Male_Growth_Curves.pdf

Male Survival and Body Weights

Male_Survival_and_Body_Weights_508c.pdf

P02 – Incidence Rates of Neoplasms by Anatomic Site

0303902_P02_Incidence_Rates_of_Neoplasms_by_Anatomic_Site.pdf

P03 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site

0303902_P03_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site.pdf

P04 – Neoplasms by Individual Animal

0303902_P04_Neoplasms_by_Individual_Animal.pdf

P05 – Incidence Rates of Neoplasms by Anatomic Site (Systemic Lesions Abridged)

0303902_P05_Incidence_Rates_of_Neoplasms_by_Anatomic_Site_Systemic_Lesions_Abridged.pdf

P08 – Statistical Analysis of Primary Tumors – No Untreated

0303902_P08_Statistical_Analysis_of_Primary_Tumors_no_untreated.pdf

P08 – Statistical Analysis of Primary Tumors – Vehicle vs Untreated Control

0303902_P08_Statistical_Analysis_of_Primary_Tumors_vehicle_vs_untreated.pdf

P09 – Non-Neoplastic Lesions by Individual Animal

0303902_P09_Non-Neoplastic_Lesions_by_Individual_Animal.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions – Vehicle vs Untreated Control

0303902_P10_Statistical_Analysis_of_Nonneoplastic_Lesions_vehicle_vs_untreated.pdf

P10 – Statistical Analysis of Non-Neoplastic Lesions – No Untreated

0303902_P10_Statistical_Analysis_of_Nonneoplastic_Lesions_no_untreated.pdf

P11 – Statistical Analysis of Survival Data

0303902_P11_Statistical_Analysis_of_Survival_Data.pdf

P14 – Individual Animal Pathology Data

0303902_P14_Individual_Animal_Pathology_Data.pdf

P17 – Neoplasms by Individual Animal (Systemic Lesions Abridged)

0303902_P17_Neoplasms_By_Individual_Animal_Systemic_Lesions_Abridged.pdf

P18 – Incidence Rates of Non-Neoplastic Lesions by Anatomic Site with Average Severity Grades

0303902_P18_Incidence_Rates_of_Nonneoplastic_Lesions_by_Anatomic_Site_with_Average_Severity_Grades.pdf

P40 – Survival Curves

0303902_P40_Survival_Curves.pdf

Triclosan In-life Observations at the Application Site

Triclosan_in_life_observations_at_the_application_site.pdf

E.1.2. Individual Animal Data

Female Individual Animal Neoplastic Pathology Data

0303902_Female_Individual_Animal_Neoplastic_Pathology_Data.xls

Female Individual Animal Non-Neoplastic Pathology Data

0303902_Female_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Female Individual Animal Survival Data

0303902_Female_Individual_Animal_Survival_Data.xls

Male Individual Animal Neoplastic Pathology Data

0303902_Male_Individual_Animal_Neoplastic_Pathology_Data.xls

Male Individual Animal Non-Neoplastic Pathology Data

0303902_Male_Individual_Animal_Nonneoplastic_Pathology_Data.xls

Male Individual Animal Survival Data

0303902_Male_Individual_Animal_Survival_Data.xls



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