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NTP Technical Report on the Toxicology Studies of Glycidamide (CASRN 5694-00-8) in F344/N NCTR Rats and B6C3F1/NCTR Mice (Drinking Water Studies)

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NTP Technical Report on the Toxicology and Carcinogenesis Studies of Glycidamide (CASRN 5694-00-8) in F344/N Nctr Rats and B6C3F1/Nctr Mice (Drinking Water Studies)

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Foreword

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

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 Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection 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 FDA Good Laboratory Practice Regulations and must meet or exceed 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 Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

The 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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This report has been reformatted to meet new NTP publishing requirements; its content has not been changed.

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Explanation of Levels of Evidence of Carcinogenic Activity

The National Toxicology Program 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, inasmuch 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 on 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 members of the Peer Review Panel who evaluated the draft *NTP Technical Report on the Toxicology and Carcinogenesis Studies of Glycidamide (CASRN 5694-00-8) in F344/N Nctr Rats and B6C3F1/Nctr Mice (Drinking Water Studies)* on October 29, 2013, are listed below. Panel members served as independent scientists, not as representatives of any institution, company, or governmental agency.

In this capacity, panel members had five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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Abstract

Glycidamide is a reactive electrophile that occurs primarily as a metabolite of acrylamide. Because acrylamide can be formed as a by-product during the cooking of starchy foods (including French fries, potato chips, and bread) and the roasting of coffee, the National Toxicology Program performed simultaneous studies to determine and compare the long-term effects of acrylamide and glycidamide in male and female F344/N Nctr rats and B6C3F1/Nctr mice. The data from the animals exposed to acrylamide formed the basis for NTP Technical Report 575. The results from the studies with glycidamide form the basis for the current report.

Two-week Study in Rats

Groups of four male and four female F344/N Nctr rats were administered 0, 0.14, 0.35, 0.70, 1.41, 3.52, or 7.03 mM glycidamide in the drinking water (0, 12.2, 30.6, 61.2, 122, 306, or 612 ppm glycidamide) for 14 days. One female rat given 7.03 mM glycidamide in the drinking water died after 12 days of treatment. Male rats administered 3.52 and 7.03 mM glycidamide weighed 84% and 60% of the control rats; female rats weighed 87% and 58% of the control rats. All male rats and one of four female rats receiving 7.03 mM glycidamide in drinking water exhibited hind limb paresis on day 14. Mild-to-moderate dilatation of the urinary bladder was observed in three of four male rats and one of four female rats given 7.03 mM glycidamide in drinking water. Mild-to-moderate degeneration of the germinal epithelium in the seminiferous tubules of the testes was noted microscopically in all male rats given 7.03 mM glycidamide in drinking water.

Two-week Study in Mice

Groups of four male and four female B6C3F1/Nctr mice were administered 0, 0.14, 0.35, 0.70, 1.41, 3.52, or 7.03 mM glycidamide in the drinking water (0, 12.2, 30.6, 61.2, 122, 306, or 612 ppm glycidamide) for 14 days. Mice administered 7.03 mM glycidamide in the drinking water for 14 days showed marked decreases in body weight (>30% compared to control mice). A single female mouse treated with 7.03 mM glycidamide in the drinking water displayed an abnormal posture and gait after 14 days of treatment. Male mice given 3.52 mM glycidamide in the drinking water for 14 days showed a modest decrease (8–13%) in body weight. There were no other adverse effects in either male or female mice administered 3.52 mM glycidamide in the drinking water for 14 days.

Three-month Study in Rats

Groups of eight male and eight female F344/N Nctr rats were administered 0.0, 0.14, 0.35, 0.70, 1.41, or 3.52 mM glycidamide in the drinking water (0, 12.2, 30.6, 61.2, 122, or 306 ppm glycidamide) for 3 months. After 3 months, male rats administered 1.41 and 3.52 mM glycidamide weighed 86% and 78% of the control rats; female rats weighed 88% and 78% of the control rats. Hind limb paresis was observed in all rats administered 3.52 mM glycidamide in the drinking water. A low incidence of peripheral neuropathy involving the sciatic nerve and axonal degeneration of the lumbar spinal cord was noted in male and female rats administered 3.52 mM glycidamide. The neuronal degenerative changes were accompanied by luminal dilation of the urinary bladder. Degeneration of the germ cells in the testes was observed in all male rats given 1.41 or 3.52 mM glycidamide. A lower incidence of this lesion was also detected in all other doses of glycidamide.

Three-month Study in Mice

Groups of eight male and eight female B6C3F1/Nctr mice were administered 0, 0.14, 0.35, 0.70, 1.41, or 3.52 mM glycidamide in the drinking water (0, 12.2, 30.6, 61.2, 122, or 306 ppm glycidamide) for 3 months. One female mouse administered 1.41 mM glycidamide died before the end of the experiment. After 3 months, the male mice given 3.52 mM glycidamide weighed 90% of the control male mice; none of the other groups had significant changes in body weight gain. Hindlimb paresis was observed in two of eight male mice administered 3.52 mM glycidamide. Peripheral neuropathy, involving primarily the sciatic nerve, was noted in male and female mice treated with 3.52 mM glycidamide. The neuronal degenerative changes were accompanied, at times, by atrophy in skeletal muscle of the hind limb and luminal dilation of the urinary bladder. Degeneration of the germ cells in the testes was observed in seven of eight male mice given 3.52 mM glycidamide.

Two-year Study in Rats

Groups of 48 male and 48 female F344/N Nctr rats were administered 0, 0.0875, 0.175, 0.35, or 0.70 mM glycidamide (0, 7.65, 15.3, 30.6, and 61.2 ppm glycidamide) in the drinking water ad libitum for 2 years resulting in average daily consumptions for the entire 2-year period of 0.39, 0.79, 1.56, and 3.34 mg glycidamide per kg body weight in male F344/N Nctr rats and 0.54, 1.08, 2.23, and 4.65 mg glycidamide per kg body weight in female F344/N Nctr rats.

There were significant dose-related decreasing trends in body weight in both male and female F344/N Nctr rats exposed to glycidamide in the drinking water. At the end of the 2-year period, F344/N Nctr rats administered 0.70 mM glycidamide weighed 79%–82% of the control groups. Food and water consumption were generally not affected by glycidamide. Male and female F344/N Nctr rats administered 0.35 and 0.70 mM glycidamide had decreased survival compared to control F344/N Nctr rats.

In male F344/N Nctr rats, the incidence of epididymal malignant mesothelioma, testicular malignant mesothelioma, and combined epididymal or testicular malignant mesothelioma was increased significantly in the 0.35 and 0.70 mM glycidamide dose groups. Malignant schwannoma of the heart, squamous cell papilloma of the tongue, combined squamous cell papilloma or carcinoma of the oral mucosa or tongue, follicular cell adenoma, follicular cell carcinoma, and combined follicular cell adenoma or carcinoma of the thyroid gland, and mononuclear cell leukemia were increased significantly in the 0.70 mM glycidamide dose group.

In female F344/N Nctr rats, the incidence of mammary gland fibroadenoma was increased significantly in all glycidamide dose groups. The incidence of follicular cell adenoma of the thyroid gland increased at 0.70 mM and the incidence of combined follicular cell adenoma or carcinoma of the thyroid gland was increased significantly in the 0.175, 0.35, and 0.70 mM glycidamide dose groups; the incidence of clitoral gland carcinoma was increased significantly in the 0.35 and 0.70 mM glycidamide dose groups; and the incidence of combined squamous cell papilloma or carcinoma of the oral mucosa or tongue, squamous cell papilloma of the forestomach, and mononuclear cell leukemia was increased significantly at 0.70 mM glycidamide.

Two-year Study in Mice

Groups of 48 male and 48 female B6C3F1/Nctr mice were administered 0, 0.0875, 0.175, 0.35, or 0.70 mM glycidamide (0, 7.65, 15.3, 30.6, and 61.2 ppm glycidamide) in the drinking water ad libitum for 2 years. Concentrations of 0.0875, 0.175, 0.35, and 0.70 mM glycidamide resulted

in an average daily consumption for the entire 2-year period of 1.20, 2.65, 5.13, and 9.55 mg glycidamide per kg body weight in male B6C3F1/Nctr mice and 1.37, 2.89, 5.64, and 12.99 mg glycidamide per kg body weight in female B6C3F1/Nctr mice.

There were no consistent body weight changes in either male or female B6C3F1/Nctr mice exposed to glycidamide. Food and water consumption were generally not affected by glycidamide, except toward the end of the study. There were dose-related decreasing trends in survival in B6C3F1/Nctr mice, with survival being significantly decreased in male B6C3F1/Nctr mice administered 0.175, 0.35, and 0.70 mM glycidamide and female B6C3F1/Nctr mice given 0.35 and 0.70 mM glycidamide.

In male B6C3F1/Nctr mice, the incidence of Harderian gland adenoma and alveolar/bronchiolar adenoma of the lung was increased significantly in all glycidamide dose groups. The incidence of squamous cell papilloma and combined squamous cell papilloma or carcinoma of the skin, and squamous cell papilloma and combined squamous cell papilloma or carcinoma of the forestomach was increased significantly at 0.70 mM glycidamide.

In female B6C3F1/Nctr mice, the incidence of Harderian gland adenoma was increased significantly in all glycidamide dose groups. Mammary gland adenoacanthoma increased at 0.70 mM and the incidence of adenocarcinoma and combined adenocarcinoma or adenoacanthoma of the mammary gland increased at 0.35 and 0.70 mM glycidamide. Fibrosarcoma of the skin increased significantly at 0.70 mM and combined fibrosarcoma or sarcoma of the skin was increased significantly at 0.35 and 0.70 mM glycidamide. The incidence of alveolar/bronchiolar adenoma of the lung and squamous cell papilloma of the forestomach was increased significantly at 0.70 mM glycidamide.

Conclusions

Under the conditions of this 2-year drinking water study, there was *clear evidence of carcinogenic activity* (see Explanation of Levels of Evidence of Carcinogenic Activity; see a summary of the peer review panel comments and the public discussion on this Technical Report in Appendix L) of glycidamide in male F344/N Nctr rats based upon increased incidences of malignant mesothelioma of the epididymis and testis tunica, malignant schwannoma of the heart, follicular cell adenoma or carcinoma of the thyroid gland, and oral cavity (oral mucosa or tongue) squamous cell neoplasms (primarily papilloma). An increased incidence of mononuclear cell leukemia may have been related to glycidamide exposure. There was *clear evidence of carcinogenic activity* of glycidamide in female F344/N Nctr rats based upon increased incidences of mammary gland fibroadenoma, oral cavity (oral mucosa or tongue) squamous cell neoplasms (primarily papilloma), follicular cell adenoma or carcinoma of the thyroid gland, and carcinoma of the clitoral gland. Increased incidences of squamous cell papilloma of the forestomach and mononuclear cell leukemia were also considered to be related to glycidamide exposure.

There was *clear evidence of carcinogenic activity* of glycidamide in male B6C3F1/Nctr mice based upon increased incidences of adenoma of the Harderian gland, alveolar/bronchiolar adenoma of the lung, squamous cell neoplasms (primarily papilloma) of the skin and forestomach. There was *clear evidence of carcinogenic activity* of glycidamide in female B6C3F1/Nctr mice based upon increased incidences of adenoma of the Harderian gland, alveolar/bronchiolar adenoma of the lung, adenoacanthoma and adenocarcinoma of the mammary gland, squamous cell papilloma of the forestomach, and malignant mesenchymal

neoplasms of the skin. The occurrence of granulosa cell tumors of the ovary may have been related to glycidamide exposure.

In F344/N Nctr rats, exposure to glycidamide was associated with increased incidence of brain gliosis (males and females), exfoliated germ cells within the epididymis (males), hepatocyte degeneration (males), liver necrosis (males), bone marrow hyperplasia (females), axonal degeneration of the lumbar spinal cord (females), and uterine endometrial hyperplasia (females).

In B6C3F1/Nctr mice, exposure to glycidamide was associated with increased incidences of cataracts (males and females), corneal inflammation (males and females), forestomach squamous cell hyperplasia (males and females), hematopoietic cell proliferation of the spleen (males and females), preputial gland lesions (degeneration, ductal dilatation, inflammation) (males), ovarian cysts (females), hepatic angiectasis and necrosis (females), and axonal degeneration of the cervical spinal cord (females).

The results of this bioassay, when compared to those previously reported for acrylamide, indicate that acrylamide is efficiently metabolized to glycidamide in both sexes of both species. Based upon the concordance of tumor sites between the two bioassays, the data also indicate that carcinogenic activity of acrylamide is due to its metabolic conversion to glycidamide.

Synonyms: 2,3-epoxypropanamide; glycidic acid amide; oxirane-2-carboxamide; oxiranecarboxamide

Summary of the Two-year Carcinogenesis Study of Glycidamide

	Male F344/N Nctr Rats	Female F344/N Nctr Rats	Male B6C3F1/Nctr Mice	Female B6C3F1/Nctr Mice
Doses in drinking water	0, 0.0875, 0.175, 0.35, or 0.70 mM glycidamide (0, 7.65, 15.3, 30.6, and 61.2 ppm glycidamide) ad libitum for 2 years	0, 0.0875, 0.175, 0.35, or 0.70 mM glycidamide (0, 7.65, 15.3, 30.6, and 61.2 ppm glycidamide) ad libitum for 2 years	0, 0.0875, 0.175, 0.35, or 0.70 mM glycidamide (0, 7.65, 15.3, 30.6, and 61.2 ppm glycidamide) ad libitum for 2 years	0, 0.0875, 0.175, 0.35, or 0.70 mM glycidamide (0, 7.65, 15.3, 30.6, and 61.2 ppm glycidamide) ad libitum for 2 years
Body weights	0.70 mM glycidamide exposure group weighed 82% of control group after 2 years	0.70 mM glycidamide exposure group weighed 79% of control group after 2 years	No significant body weight changes	Only sporadic changes, with magnitude ≤5% of controls
Survival rates	21/48, 18/48, 15/48, 7/48, 2/48	35/48, 26/48, 27/48, 17/48, 2/48	45/48, 41/48, 34/48, 26/48, 25/48	41/48, 42/48, 38/48, 31/48, 8/48
Nonneoplastic effects	Brain: gliosis (0/48, 1/48, 0/48, 0/47, 4/48) Epididymis: exfoliated germ cells (0/48, 1/45, 2/48, 3/47, 4/47) Liver: hepatocyte degeneration (2/47, 6/47, 6/48, 10/47, 8/47); necrosis (1/47, 5/47, 2/48, 7/47, 5/47)	Brain: gliosis (0/48, 0/48, 4/48, 4/48, 4/48) Bone marrow: hyperplasia (2/48, 6/48, 7/46, 8/47, 14/47) Spinal cord (lumbar): axonal degeneration (5/48, 6/48, 5/47, 6/48, 9/48) Uterus: endometrial hyperplasia (11/48, 17/48, 14/48, 14/48, 23/48)	Eye: cataract (1/47, 3/45, 7/46, 8/44, 17/42); corneal inflammation (0/47, 0/45, 2/46, 0/44, 8/42) Preputial gland: degeneration (4/47, 10/47, 5/46, 12/46, 9/44); ductal dilatation (0/47, 0/47, 1/46, 0/46, 4/44); inflammation (1/47, 6/47, 2/46, 3/46, 9/44) Spleen: hematopoietic cell proliferation (6/47, 6/47, 12/47, 14/46, 17/44) Stomach (forestomach): epithelial hyperplasia (5/47, 2/45, 5/48, 5/45, 12/41)	Eye: cataract (1/45, 2/44, 8/47, 8/44, 9/43); corneal inflammation (0/45, 2/44, 1/47, 3/44, 5/43) Liver: angiectasis (0/47, 0/48, 1/47, 0/46, 5/43); necrosis (0/47, 0/48, 0/47, 0/46, 5/43) Ovary: cyst (14/45, 17/47, 25/47, 22/46, 18/44)) Spinal cord (cervical): axonal degeneration (4/45, 9/44, 10/47, 9/45, 10/43) Spleen: hematopoietic cell proliferation (6/46, 10/47, 11/47, 14/47, 29/45) Stomach (forestomach): epithelial hyperplasia (4/45, 4/45, 10/47, 11/45, 5/44)

	Male F344/N Nctr Rats	Female F344/N Nctr Rats	Male B6C3F1/Nctr Mice	Female B6C3F1/Nctr Mice
Neoplastic effects	Epididymis: malignant mesothelioma (0/48, 1/45, 3/48, 10/47, 17/47) Testes: malignant mesothelioma (0/48, 1/47, 3/48, 6/47, 13/48) Epididymis or testes: malignant mesothelioma (0/48, 1/48, 3/48, 10/47, 17/48) Heart: malignant schwannoma (2/48, 3/48, 3/48, 7/47, 8/48) Oral mucosa: squamous cell papilloma (1/48, 1/48, 0/48, 2/47, 3/48); squamous cell carcinoma (1/48, 0/48, 1/48, 1/47, 0/48) Tongue: squamous cell papilloma (0/48, 1/48, 0/48, 1/47, 4/48); squamous cell carcinoma (0/48, 0/48, 1/48, 0/47, 0/48) Oral mucosa or tongue: squamous cell papilloma or carcinoma (2/48, 2/48, 2/48, 3/47, 7/48) Thyroid gland: follicular cell adenoma (2/47, 1/42, 3/48, 3/47, 8/46); follicular cell carcinoma (0/47, 2/42, 3/48, 1/47, 5/46); follicular cell adenoma or carcinoma (2/47, 3/42, 6/48, 4/47, 13/46)	Clitoral gland: carcinoma (4/48, 6/48, 7/48, 11/48, 14/47) Mammary gland: fibroadenoma (16/48, 26/48, 35/48, 33/48, 36/48) Oral mucosa: squamous cell papilloma (1/48, 1/48, 2/48, 0/48, 4/48); squamous cell carcinoma (0/48, 0/48, 0/48, 1/48, 2/48) Tongue: squamous cell papilloma (0/48, 1/48, 0/48, 1/48, 0/48); squamous cell carcinoma (0/48, 0/48, 0/48, 0/48, 1/48) Oral mucosa or tongue: squamous cell papilloma or carcinoma (1/48, 2/48, 2/48, 2/48, 7/48) Stomach: forestomach squamous cell papilloma (0/48, 1/48, 0/48, 0/47, 3/46) Thyroid gland: follicular cell adenoma (0/48, 3/48, 3/46, 1/46, 5/47); follicular cell carcinoma (0/48, 0/48, 2/46, 3/46, 3/47); follicular cell adenoma or carcinoma (0/48, 3/48, 5/46, 4/46, 8/47) Mononuclear cell leukemia (14/48, 11/48, 21/48, 19/48, 27/48)	Harderian gland: adenoma (3/47, 17/47, 23/47, 32/46, 42/47) Lung: alveolar/bronchiolar adenoma (0/47, 7/46, 7/47, 13/47, 17/47) Skin: squamous cell papilloma (0/47, 1/48, 2/47, 1/47, 8/46); squamous cell carcinoma (0/47, 0/48, 0/47, 0/47, 2/46); squamous cell papilloma or carcinoma (0/47, 1/48, 2/47, 1/47, 9/46) Stomach: forestomach squamous cell papilloma (0/47, 2/45, 3/48, 2/45, 10/41); forestomach squamous cell carcinoma (0/47, 0/45, 0/48, 0/45, 2/41); forestomach squamous cell papilloma or carcinoma (0/47, 2/45, 3/48, 2/45, 12/41)	Harderian gland: adenoma (2/45, 19/47, 20/47, 24/46, 40/46) Lung: alveolar/bronchiolar adenoma (3/46, 5/48, 3/47, 7/47, 9/44) Mammary gland: adenoacanthoma (0/45, 0/48, 0/47, 1/47, 8/45); adenocarcinoma (1/45, 1/48, 2/47, 9/47, 11/45); adenoacanthoma or adenocarcinoma (1/45, 1/48, 2/47, 9/47, 18/45) Skin: fibrosarcoma (0/45, 1/48, 2/47, 2/47, 9/45); sarcoma (0/45, 0/48, 1/47, 3/47, 3/45); fibrosarcoma or sarcoma (0/45, 1/48, 3/47, 5/47, 12/45) Stomach: forestomach squamous cell papilloma (1/45, 1/45, 1/47, 5/45, 9/44)

	Male F344/N Nctr Rats	Female F344/N Nctr Rats	Male B6C3F1/Nctr Mice	Female B6C3F1/Nctr Mice
Equivocal findings	Mononuclear cell leukemia: (21/48, 26/48, 27/48, 27/47, 31/48)	None	None	Ovary: granulosa cell tumor—benign or malignant (0/45, 0/47, 0/47, 3/46, 3/44)
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence

Introduction

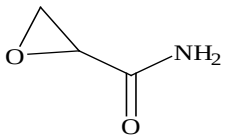


Figure 1. Glycidamide (CASRN 5694-00-8; Chemical Formula: $C_3H_5NO_2$; Molecular Weight: 87.08)

Synonyms: 2,3-epoxypropanamide; glycidic acid amide; oxirane-2-carboxamide; oxiranecarboxamide.

Chemical and Physical Properties

Glycidamide (Figure 2) is a pale orange, hygroscopic crystalline solid. It is soluble in acetone, dichloromethane, and water, and has a melting point of 32° to 34°C (recrystallized from acetone)[1].

Production, Use, and Human Exposure

Glycidamide is prepared by the epoxidation of acrylonitrile under basic conditions[1]. The primary use of glycidamide is as an intermediate in organic synthesis, for example, as a synthetic intermediate in the production of dyes and plasticizers[2].

Glycidamide is a major metabolite of the α,β-unsaturated amide acrylamide (reviewed in Shipp et al.[3]; NTP[4]). As a consequence, the major source of human exposure to glycidamide occurs through exposure to acrylamide either in occupational situations, through the diet, or by the use of tobacco products (reviewed in Shipp et al.[3]; NTP[4]). Glycidamide has also been reported to be present in certain foods, at a level of less than 1% that of acrylamide[5].

Biological and Toxicological Properties

Absorption, Distribution, Metabolism, and Excretion in Experimental Animals

The absorption, distribution, metabolism, and excretion of acrylamide in experimental animals have been reviewed[3; 4].

The toxicokinetic parameters of glycidamide have been determined in B6C3F1 mice and F344 rats after a single intravenous or oral (gavage) administration[6; 7]. After intravenous injection of glycidamide to B6C3F1 mice, the elimination half-life ($t_{1/2}$) was 1.0–1.1 hr, the time-serum concentration curve from zero to infinity (AUC) was 2.9 μM hr, and the volume of distribution (V_d) was 0.70–0.74 ml/g[6] (Table 1). In B6C3F1 mice treated by gavage with glycidamide, the maximum concentration (C_{max}) was observed at the initial sampling point of 15 min, and the $t_{1/2}$ and AUC values were similar to those determined in B6C3F1 mice injected intravenously[6] (Table 1).

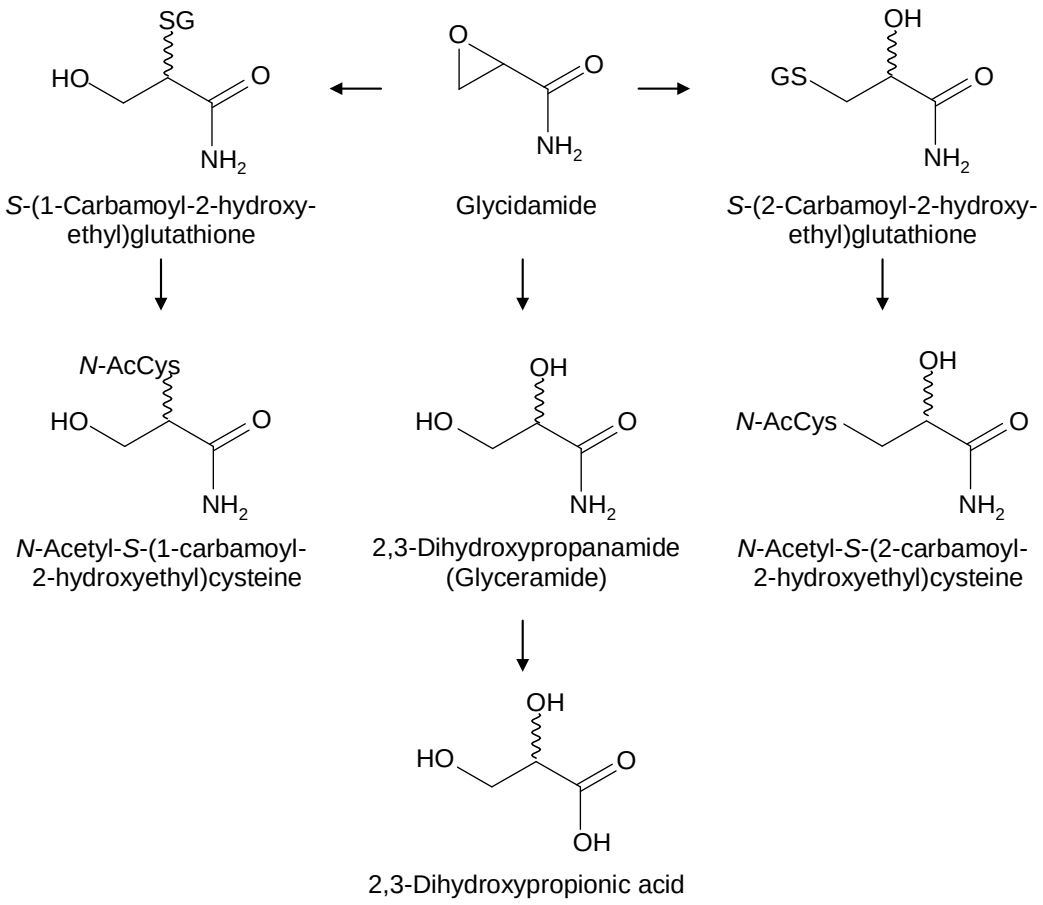


Figure 2. Metabolites of Glycidamide

Table 1. Toxicokinetic Parameters for Glycidamide Administered to B6C3F1 Mice and F344 Rats[a]

	B6C3F1 Mice				F344 Rats			
	Intravenous		Gavage		Intravenous		Gavage	
	Male	Female	Male	Female	Male	Female	Male	Female
Glycidamide AUC[b] (μM hr)	2.9	2.9	3.4	3.3	2.8	3.3	2.8	3.8
Glycidamide $t_{1/2}$ absorption (hr)	–	–	–	–	–	–	0.31	1.8
Glycidamide $t_{1/2}$ elimination (hr)	1.1	1.0	1.1	1.2	1.3	1.4	1.7	1.8
Glycidamide V_d (ml/g)	0.74	0.70	–	–	0.77	0.68	–	–
Glycidamide C_{max} (μM)	–	–	1.5	1.6	–	–	0.70	0.92

[a]The pharmacokinetic parameters were determined after a single administration of 0.12 mg/kg glycidamide (1.4 μmol/kg). Adapted from Doerge et al.[6; 7].
[b]The abbreviations used are: AUC, area under the curve; $t_{1/2}$, half-life; V_d, volume of distribution; C_{max}, maximum concentration.

Intravenous administration of glycidamide to F344 rats resulted in V_d and AUC values similar to those observed in B6C3F1 mice and a slightly longer elimination $t_{1/2}$[7] (Table 1). After oral gavage of glycidamide to F344 rats, the C_{max} values occurred at 1 hr and were approximately 50% of those observed in B6C3F1 mice, the AUC values were comparable to those in B6C3F1 mice, while the $t_{1/2}$ values were slightly longer[7] (Table 1).

Glycidamide reacts with glutathione to give *S*-(1-carbamoyl-2-hydroxyethyl)glutathione and *S*-(2-carbamoyl-2-hydroxyethyl)glutathione, which after further metabolic processing yield *N*-acetyl-*S*-(1-carbamoyl-2-hydroxyethyl)cysteine and *N*-acetyl-*S*-(2-carbamoyl-2-hydroxyethyl)cysteine[8] (Figure 2). Glycidamide also undergoes hydrolysis to form 2,3-dihydroxypropanamide (glyceramide) and subsequently 2,3-dihydroxypropionic acid[8; 9] (Figure 2). The cysteine conjugates, glyceramide, and 2,3-dihydroxypropionic acid have been detected in the urine of mice and rats treated with acrylamide[8-13]. The formation of these metabolites does not appear to have been examined in experimental animals administered glycidamide.

Incubation of primary liver hepatocytes from male Sprague-Dawley rats with 0.3, 1, or 3 mM glycidamide resulted in a rapid statistically significant decrease in the glutathione content of the cells[14]. Glutathione depletion was not observed with 0.1 mM glycidamide and the effect with glycidamide was more pronounced than that observed with an equimolar concentration of acrylamide. The depletion of glutathione could be prevented partially by co-incubation with N-acetylcysteine or methionine.

A number of DNA adducts have been characterized from the reaction of glycidamide with DNA and/or deoxynucleosides. These include N7-(2-carbamoyl-2-hydroxyethyl)guanine (N7-GA-Gua; from the depurination of N7-(2-carbamoyl-2-hydroxyethyl)deoxyguanosine), N3-(2-carbamoyl-2-hydroxyethyl)adenine (N3-GA-Ade; from the depurination of N3-(2-carbamoyl-2-hydroxyethyl)deoxyadenosine), N1-(2-carboxy-2-hydroxyethyl)deoxyadenosine, N^6-(2-carboxy-2-hydroxyethyl)deoxyadenosine (from a Dimroth rearrangement of N1-(2-carboxy-2-

hydroxyethyl)deoxyadenosine), N3-(2-carbamoyl-2-hydroxyethyl)thymidine, N3-(2-carboxy-2-hydroxyethyl)deoxycytidine, N1-(2-carboxy-2-hydroxyethyl)deoxyguanosine, N1-(2-carbamoyl-2-hydroxyethyl)deoxyguanosine, N1,N^6-(2-hydroxypropanoyl)deoxyadenosine, and N3,N^4-(2-hydroxypropanoyl)deoxycytidine[15-20] (Figure 3).

N7-GA-Gua and N3-GA-Ade have been detected in mice and rats treated with glycidamide[6; 7; 17; 21-23]. Both adducts are typically detected in all tissues examined, with N7-GA-Gua being formed to a 100-fold greater extent than N3-GA-Ade[17; 22; 23], a ratio that corresponds to that observed in DNA reacted with glycidamide in vitro[17]. N7-GA-Gua and N3-GA-Ade have been found in Chinese hamster lung V79 cells[24] and L5178Y/$Tk^{+/-}$ mouse lymphoma cells[25] treated in vitro with glycidamide, with the ratio again being ~100:1 (N7-GA-Gua:N3-GA-Ade). N1-(2-Carboxy-2-hydroxyethyl)deoxyadenosine has been reported in DNA from cells treated in vitro with glycidamide; it was not detected in vivo[18].

Glycidamide reacts with cysteine residues in hemoglobin and other proteins[26; 27]. After hydrolysis with 6 N HCl, the adduct is released as *S*-(2-carboxy-2-hydroxyethyl)cysteine (Figure 4). Glycidamide also reacts with the N-terminal valine of hemoglobin to give (after acid hydrolysis) *N*-(2-carboxy-2-hydroxyethyl)valine[28] (Figure 4).

N-(2-Carboxy-2-hydroxyethyl)valine was detected in hemoglobin isolated from B6C3F1 mice and F344 rats receiving single doses of glycidamide by either intravenous or oral (gavage) administration. In B6C3F1 mice, the levels of *N*-(2-carboxy-2-hydroxyethyl)valine were comparable by both routes of administration and did not differ between sexes. With F344 rats, the adduct levels were also comparable by both routes of administration, but were somewhat higher in females as compared to males[22].

In Sprague-Dawley rats administered a single intraperitoneal dose of glycidamide (0–100 mg per kg body weight), the formation of glycidamide hemoglobin adducts (measured as *S*-(2-carboxy-2-hydroxyethyl)cysteine) varied linearly with the dose[26]. Likewise, there were linear correlations between the serum AUC for glycidamide in B6C3F1 mice and F344 rats and the levels of glycidamide hemoglobin adducts (measured as *N*-(2-carboxy-2-hydroxyethyl)valine) and between the levels of glycidamide hemoglobin adducts (also measured as *N*-(2-carboxy-2-hydroxyethyl)valine) and the levels of N7-GA-Gua in hepatic DNA of B6C3F1 mice and F344 rats[22].

Absorption, Distribution, Metabolism, and Excretion in Humans

The absorption, distribution, metabolism, and excretion of acrylamide in humans have been reviewed[3; 4].

The placental transfer of glycidamide has been investigated in vitro using perfused human placentas. When incubations were conducted with 5 μg per ml glycidamide (60 μM glycidamide), the concentration after 4 hr in the fetal perfusate was nearly identical to that in the maternal perfusate. The transfer index was 33%, a value similar to that observed with acrylamide and the positive control antipyrine[29; 30].

Glycidamide was not detected in perfusion samples or placental tissue samples from incubations conducted with acrylamide. N1-(2-Carboxy-2-hydroxyethyl)deoxyadenosine was not detected in perfused placental tissue; the presence of N7-GA-Gua or N3-GA-Ade was not assessed[29]. N7-

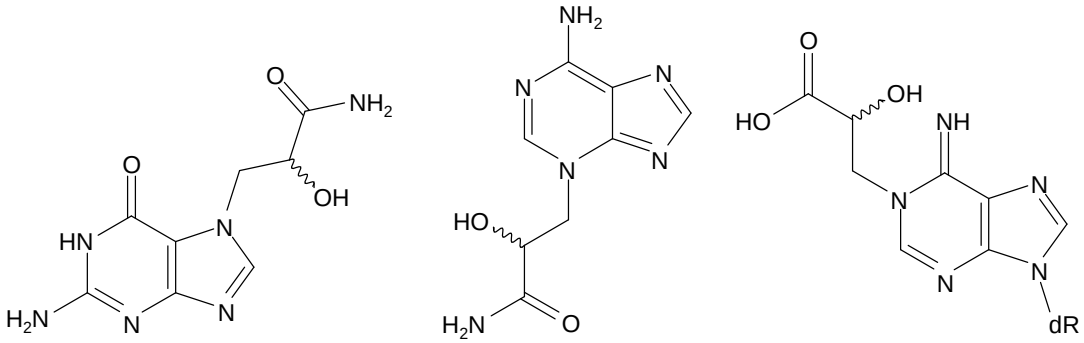
GA-Gua has been reported to be present in human lymphoid TK6 cells incubated in vitro with glycidamide[31].

Toxicity in Experimental Animals

Glycidamide is neurotoxic in experimental animals. Male Sprague-Dawley rats injected intraperitoneally daily for eight days with 50 or 100 mg glycidamide per kg body weight had a significant decrease in body weight and a decreased performance on a rotarod apparatus (at only the 100 mg glycidamide per kg body weight dose). Hind limb splay, a response observed with comparable doses of acrylamide, was not apparent[32].

In a subsequent study, male Sprague-Dawley rats received subcutaneous injections of 15.3, 30.6, or 61.3 mg glycidamide per kg body weight (0.18, 0.35, or 0.70 mMol glycidamide per kg body weight) for 39 days. None of the animals displayed any signs of neurotoxicity when monitored for 70 days after the initial injection. When male Sprague-Dawley rats were injected intraperitoneally with 0.70 mMol glycidamide per kg body weight per day, all animals developed hind limb paralysis by 13 days of treatment[33].

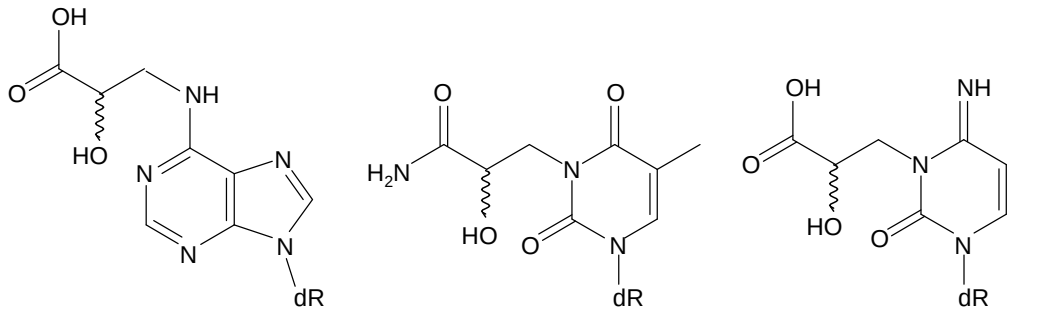
In a further experiment, male Sprague-Dawley rats were treated daily for 8 days by intraperitoneal injection with 50 or 100 mg glycidamide per kg body weight. Rats administered 100 mg glycidamide per kg body weight had a significant decrease in body weight, decreased performance on a rotarod apparatus, and decreased creatine kinase activity in the sciatic nerve, tibial nerve, and whole brain. The decrease in creatine kinase activity was also observed at 50 mg glycidamide per kg body weight[34]. A decrease in glyceraldehyde-3-phosphate dehydrogenase activity has also been reported in peripheral nerves of rats treated intraperitoneally for 8 days with 50 or 100 mg glycidamide per kg body weight[35].



N7-(2-Carbamoyl-2-hydroxy-ethyl)guanine (N7-GA-Gua)

N3-(2-Carbamoyl-2-hydroxy-ethyl)adenine (N3-GA-Ade)

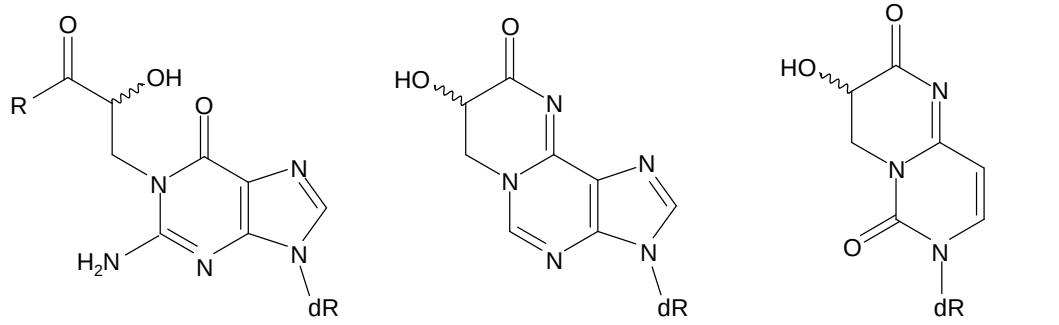
N1-(2-Carboxy-2-hydroxy-ethyl)deoxyadenosine



N^6-(2-Carboxy-2-hydroxy-ethyl)deoxyadenosine

N3-(2-Carbamoyl-2-hydroxy-ethyl)thymidine

N3-(2-Carboxy-2-hydroxy-ethyl)deoxycytidine



N1-(2-Carboxy-2-hydroxyethyl)-deoxyguanosine (R = OH)
N1-(2-Carbamoyl-2-hydroxyethyl)-deoxyguanosine (R = NH_2)

N1,*N*^{6}-(2-Hydroxypropanoyl)-deoxyadenosine

N3,*N*^{4}-(2-Hydroxypropanoyl)-deoxycytidine

Figure 3. DNA Adducts of Glycidamide

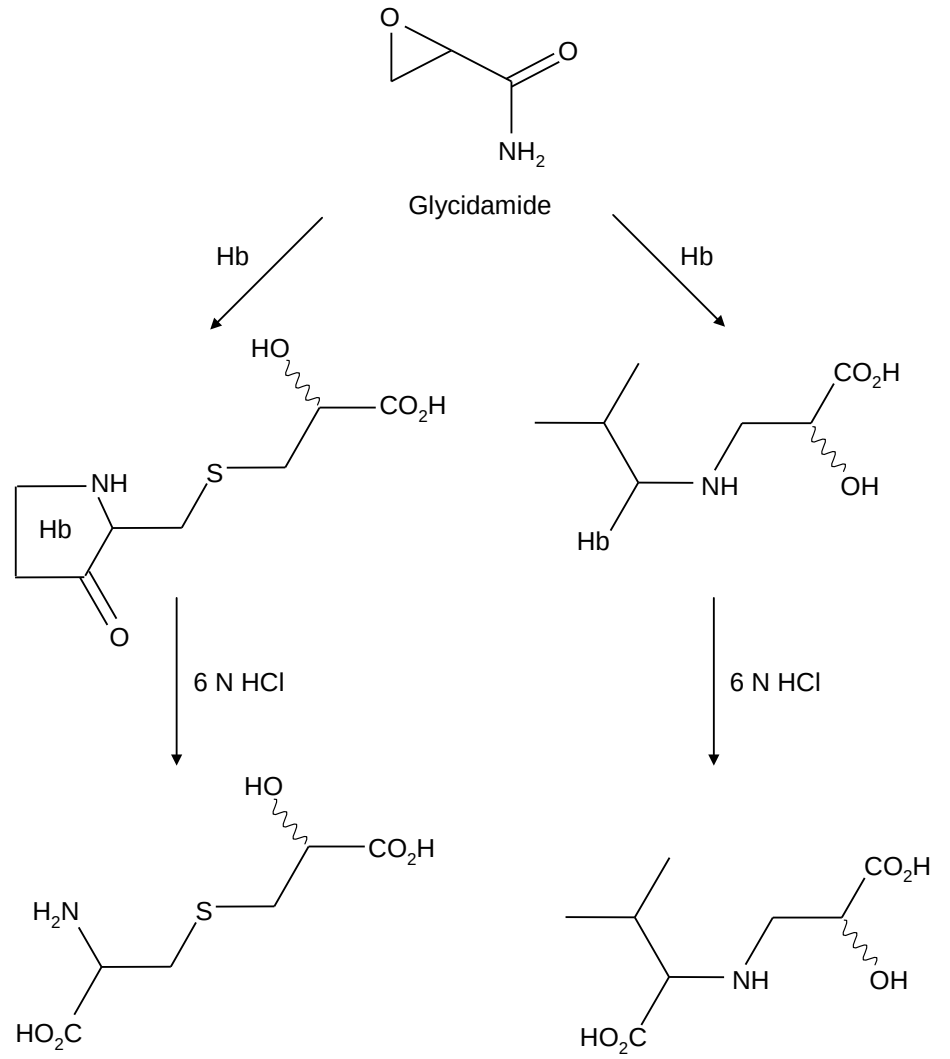


Figure 4. Hemoglobin (Hb) Adducts of Glycidamide

Toxicity in Humans

Although the toxicity of acrylamide in humans is well documented (reviewed in Shipp et al.[3]; NTP[4]), there are no toxicity data in humans from the direct exposure to glycidamide.

Reproductive Toxicity and Teratogenicity in Experimental Animals

Male Sprague-Dawley rats treated daily for 8 days with 50 mg glycidamide per kg body weight had significant decreases in testis protein content, epididymis weight, vas deferens sperm count, and sperm cell viability when measured one day after the final dose. Total testis weight was not affected[32].

Male B6D2F1 mice were treated daily for 8 days with intraperitoneal injections of 61 mg glycidamide per kg body weight per day. One day after the last injection, sperm was isolated and used in an in vitro fertilization experiment with oocytes from female B6C3F1 mice. The expression of 39 genes involved in the response to DNA damage and in embryo development was assessed in zygotes and embryos at 1-, 2-, 4-, and 8-cell stages. Fourteen genes were significantly affected by glycidamide treatment, with there being decreased expression at early time points and increased expression at later time points[36].

Reproductive Toxicity and Teratogenicity in Humans

There are no data pertaining to the reproductive toxicity and teratogenicity of glycidamide in humans.

Carcinogenicity in Experimental Animals

The effect of perinatal exposure to glycidamide was investigated in C57BL/6J *Min*/+ mice, a strain susceptible to intestinal neoplasia, and their wild type littermates. In one experiment, C57BL/6J *Min*/+ mice and their wild type C57BL/6J littermates were treated subcutaneously at one and two weeks after birth with 10 or 50 mg glycidamide per kg body weight. When assessed at eight weeks of age, C57BL/6J *Min*/+ mice (males and females combined) had a slight but significant dose-related induction of small intestinal tumors, with the increase being significant at 50 mg glycidamide per kg body weight. The wild-type C57BL/6J littermates were assessed at 32 weeks of age. At this time, mice administered 50 mg glycidamide per kg body weight had a significant increase in intestinal lesions (small intestinal tumors, and either flat or classical aberrant crypt foci)[37].

In a second experiment, the mothers of one group of C57BL/6J *Min*/+ mice were exposed to 50 mg (0.57 mMol) glycidamide per kg body weight 1 week before giving birth, a second group was treated at the same dose level 1 and 2 weeks after birth, and a third group received both the prenatal and postnatal treatments. In addition, the mothers of one group of wild type littermates were treated with 50 mg glycidamide per kg body weight 1 week before giving birth and then their pups were treated weekly from weeks 5 to 11 with 50 mg glycidamide per kg body weight; a second group was treated at weeks 1, 2, and 5 to 11 of age, and a third group received both the prenatal and postnatal treatments. When assessed at 12 weeks of age, there was a significant increase in colonic tumors in male C57BL/6J *Min*/+ mice treated prenatally and postnatally. None of the other treatments caused an increase in the tumor incidence in either C57BL/6J *Min*/+ mice or their wild type littermates[37].

Male B6C3F1 mice were injected intraperitoneally on postnatal days 1, 8, and 15 with 0.0, 0.14, or 0.70 mMol glycidamide per kg body weight per day and tumorigenicity was assessed after 1 year. The only treatment-related neoplasms involved the liver. The incidence of combined hepatocellular adenoma or carcinoma was 3.8% in the control group, 8.3% in the 0.14 mMol glycidamide per kg body weight group, and 71.4% in the 0.70 mMol glycidamide per kg body weight group. The hepatic tumor incidence in the 0.70 mMol glycidamide per kg body weight group was significantly different from the control group. Analysis of the hepatocellular tumors indicated that the increased incidence observed in mice administered 0.70 mMol glycidamide per kg body weight was associated with A→G and A→T mutations at codon 61 of the H-*ras* oncogene[38].

Carcinogenicity in Humans

There are no data pertaining to the carcinogenicity of glycidamide in humans.

Genetic Toxicity

Glycidamide is a well-documented genotoxicant, giving positive responses in vitro and in vivo in a variety of assays and test systems in somatic and germinal cells measuring gene mutations, DNA damage, and chromosomal aberrations. A brief summary of the literature follows.

Bacterial Mutagenesis Assays

Glycidamide is mutagenic in *Salmonella typhimurium* base-substitution strains TA100 and TA1535 in the absence of a rat liver S9 mix, suggesting that glycidamide is a direct-acting mutagen; the addition of S9 had no effect upon the mutagenicity of glycidamide in strain TA1535 but increased the number of revertants in strain TA100[39]. In another study, glycidamide was mutagenic in strain TA100 in the presence of S9; it did not appear to have been tested in the absence of S9[40]. Glycidamide also induced the *umuC* gene, a gene that contributes to post-replication DNA repair, in *Salmonella typhimurium* TA1535/pSK1002 in the absence of S9[31].

Glycidamide was not mutagenic in the fluctuation test with *Klebsiella pneumoniae* when tested at doses up to 50 mM[41].

In Vitro Mammalian Cell Gene Assays

The mutagenicity of glycidamide has been assessed in L5178Y/*Tk*$^{+/-}$ mouse lymphoma cells. Positive responses, in the absence of metabolic activation, were obtained at concentrations of 2.5 mM[42] and 2–4 mM[25]. The induction of mutations was associated with a dose-dependent formation of N7-GA-Gua and N3-GA-Ade DNA adducts and was attributed to a clastogenic mode of action (i.e., loss of heterozygosity)[25]. An increase in mutations was not detected in the presence of an external activation system[42].

Glycidamide induced a dose-dependent increase in the mutant frequency at the hypoxanthine-guanine phosphoribosyltransferase (*Hprt*) gene of Chinese hamster lung V79 cells at concentrations of 0.8–2.0 mM[43-45]. An increased *Hprt* mutant frequency was detected in Chinese hamster ovary cells that had been treated with ~1–4 mM glycidamide[46]. Glycidamide also induced chromosomal aberrations at concentrations of 0.25–1.0 mM and sister chromatid exchanges at concentrations of 10–1000 μM in Chinese hamster lung V79 cells. These

cytogenetic alterations were correlated with the formation of N7-GA-Gua and N3-GA-Ade DNA adducts[24].

Incubation of Big Blue® mouse embryo fibroblasts with 50 nM to 5 mM glycidamide resulted in a dose-dependent increase in the mutant frequency at the *cII* transgene. The increase in mutant frequency was attributed primarily to G→T transversion mutations, along with A→G transition and G→C transversion mutations[47].

Glycidamide induced a dose-related increase in the mutant frequency of the thymidine kinase (*TK*) gene of human lymphoblastoid TK6 cells at concentrations of 0.6–2.5 mM, with the increase being statistically significant at 2.5 mM glycidamide; the increase in the mutant frequency was due primarily to point mutations[48]. Additional endpoints measured in the TK6 cells included the induction of micronuclei, which was significantly increased at 2.5 mM glycidamide, and DNA damage, as measured by the comet assay, which was significantly increased at concentrations ≥0.6 mM glycidamide.

Incubation of human peripheral blood lymphocytes with 0.3–3 mM glycidamide[43] or 1–10 mM glycidamide[40] resulted in a dose dependent increase in DNA damage, as measured by the comet assay. An increase in micronuclei was not apparent in these cells under similar treatment conditions[43]. When comet assays were performed in the presence of formamidopyrimidine DNA glycosylase (Fpg), a bacterial enzyme that allows for assessment of oxidative DNA damage, an increase in DNA damage was observed at concentrations as low as 10 μM glycidamide[45]. In another study, human peripheral blood lymphocytes, mouse peripheral blood lymphocytes, and mouse testicular cells were incubated with 0–5 mM glycidamide, and strand breaks and alkali-labile sites were assessed by the comet assay. The maximum response was again observed when assays were conducted in the presence of Fpg and yielded effective concentrations (EC_{10}) of 3.7 μM in human peripheral blood lymphocytes, 8.6 μM in mouse peripheral blood lymphocytes, and 13.5 μM in mouse testicular cells[49]. An increased response in a comet assay, conducted in the presence of Fpg, was also reported for Chinese hamster lung V79 cells that had been incubated with 10–300 μM glycidamide[44]. An increase in DNA damage, as indicated by the comet assay, also occurred in Chinese hamster lung V79 cells, human epithelial colorectal adenocarcinoma cells (Caco-2), and primary hepatocytes from male Wistar rats exposed to ≥30, ≥300, and ≥60 μM glycidamide, respectively[50].

When glycidamide was incubated with primary rat liver hepatocytes at concentrations up to 4 mM, there was not an increase in unscheduled DNA synthesis in one experiment[42]; however, in a subsequent study, incubation with 1 or 10 mM glycidamide, but not 0.01 or 0.1 mM glycidamide, resulted in a significant increase in unscheduled DNA synthesis in hepatocytes from male F344 rats[51]. The induction of unscheduled DNA synthesis was also observed in primary human mammary epithelial cells treated with 1 or 10 mM glycidamide[51].

In Vivo Mammalian Cell Assays

A single intraperitoneal injection of 125 mg glycidamide per kg body weight produced positive results in the dominant lethal mutation test when assessed in germ cells from male (C3H/RL × 101/RL)F_1 mice. Likewise, a single intraperitoneal injection of 100 mg glycidamide per kg body weight caused a significant increase in reciprocal translocations in germ cells of

male mice. In addition, a single intraperitoneal injection with 150 mg glycidamide per kg body weight induced unscheduled DNA synthesis in mouse sperm at the early spermatid stage[52].

Male and female B6C3F1/*Tk*$^{+/-}$ and B6C3F1/*Tk*$^{+/+}$ mice were treated intraperitoneally on postnatal days 1, 8, and 15 or postnatal days 1 to 8 with 0.14 or 0.70 mMol glycidamide per kg body weight per day. One day after the final dose, DNA adduct levels and peripheral blood micronuclei were measured in B6C3F1/*Tk*$^{+/+}$ mice, and three weeks after the last treatment, *Hprt* and *Tk* mutant frequencies were assessed in spleen lymphocytes from B6C3F1/*Tk*$^{+/-}$ mice. The administration of 0.70 mMol glycidamide per kg body weight on postnatal days 1, 8, and 15 produced an increase in mutant frequency at the *Hprt* gene in splenic T-lymphocytes of B6C3F1/*Tk*$^{+/-}$ mice and an increased frequency of micronucleated reticulocytes and micronucleated normochromatic erythrocytes in peripheral blood of B6C3F1/*Tk*$^{+/+}$ mice. The administration of 0.14 mMol glycidamide per kg body weight on postnatal days 1–8 was associated with an increased mutant frequency at the *Tk* gene in splenic T-lymphocytes of B6C3F1/*Tk*$^{+/-}$ mice and an increased frequency of micronucleated normochromatic erythrocytes in peripheral blood from B6C3F1/*Tk*$^{+/+}$ mice. Molecular analysis indicated that the glycidamide-induced increases in the *Tk* mutant frequency were due, in part, to loss of heterozygosity[23]. A dose-related increase in micronucleated polychromatic erythrocytes has also been reported in male CBA mice receiving a single intraperitoneal injection of 0.18, 0.35, or 0.70 mMol glycidamide per kg body weight[53].

Male and female Big Blue® mice administered 7.0 mM glycidamide (600 ppm glycidamide) in the drinking water for four weeks had an increased mutant frequency at the *Hprt* gene in splenic T-lymphocytes, an increased mutant frequency at the *cII* transgene in liver, and an increased frequency of micronucleated reticulocytes in peripheral blood samples. An increased *Hprt* mutant frequency in splenic T-lymphocytes was also observed in mice given 1.4 mM glycidamide (120 ppm glycidamide) for four weeks. The increased mutant frequency in the *cII* transgene in liver was associated with G→T transversion mutations and also with −1/+1 frameshift mutations in a sequence of six guanine residues[54]. A subsequent report indicated that there was an increased mutant frequency in the *cII* transgene from the testes of these mice[55].

Male and female Big Blue® rats given 1.4 mM glycidamide (120 ppm) in the drinking water for 60 days had an increased mutant frequency at the *Hprt* gene in splenic T-lymphocytes, and increased mutant frequencies at the *cII* transgene in the bone marrow and thyroid gland, but not in the testis, mammary gland, or liver. No increase in mutant frequency was observed at the *cII* transgene in the livers of either sex, or in the testes of male rats or mammary gland of female rats, and no increase was detected in the frequency of micronucleated reticulocytes in either sex when measured 24 hr after the treatment ended[56]. The lack of a micronucleus response in these rats was somewhat surprising given the clear responses for this endpoint in other studies, and may be due to the lower doses used by Mei et al.[56] as well the lower sensitivity of rats to glycidamide-induced micronucleus formation[53].

Study Rationale

Acrylamide has recently been detected in roasted coffee and many baked and fried starchy foods. Existing data indicated that acrylamide is carcinogenic; however, the Center for Food Safety and Applied Nutrition, FDA, desired a modern, more definitive bioassay to perform a quantitative risk assessment. Consequently the FDA nominated acrylamide for evaluation by the NTP.

Acrylamide was hypothesized to be a genotoxic carcinogen as a result of metabolic conversion to glycidamide, which reacts with DNA. Since the metabolic conversion of acrylamide to glycidamide occurs to a greater extent in mice as compared to rats[8; 21], mice were hypothesized to be more sensitive than rats to the carcinogenic effects of acrylamide. To test these hypotheses and to provide data for a meaningful risk assessment, studies were conducted simultaneously to compare the extent and types of tumors in B6C3F1/Nctr mice and F344/N Nctr rats treated chronically with either acrylamide or glycidamide. The data from the animals exposed to acrylamide formed the basis for NTP Technical Report 575[4]. The results from the studies with glycidamide form the basis for the current report.

Materials and Methods

Procurement and Characterization

Glycidamide

Glycidamide (oxirane-2-carboxamide; $C_3H_5NO_2$; molecular weight 87.08) was purchased from Toronto Research Chemicals, North York, Ontario, Canada (Lot # 4AQL-43-5). The identity and purity of the chemical was assessed at the National Center for Toxicological Research (NCTR) by gas chromatography coupled with electron impact mass spectrometry (GC/EI-MS), nuclear magnetic resonance (NMR) spectrometry, and gas chromatography using flame ionization detection (GC-FID).

GC/EI-MS of the glycidamide indicated a major component with the proper mass (*m/z* = 87) and fragments (*m/z* = 86, 71, 70, 69, 59, 57, 44, 43, 42, and 41) consistent with the proposed structure. The purity was estimated to be >99%. ^{1}H and ^{13}C NMR spectra were consistent with the structure of glycidamide, and based upon the ^{1}H NMR spectra, the purity was estimated to be 98.5%. Identified impurities included acrylamide (0.7%), methylene chloride (0.13%), and other aliphatics (0.6%). GC-FID of the glycidamide indicated one major peak that accounted for 98.4% of the material and three minor peaks, with areas of 0.3, 1.1, and 0.2%.

Preparation and Analysis of Dose Formulations

The stability of glycidamide in drinking water was assessed at a concentration of 30.6 μg per ml for a period of 35 days at room temperate in the absence of light. From 0–21 days, the recovery of glycidamide varied between 97.7% and 118%; at 28 and 35 days, the recovery was 73.7% and 66.5%, respectively.

For the 2-week study, glycidamide drinking water solutions were prepared weekly for treating animals. The target concentrations were 12.2, 30.6, 61.2, 122, 306, and 612 μg glycidamide per ml corresponding to 0.14, 0.35, 0.70, 1.41, 3.52, and 7.03 mM glycidamide. Concentrations were deemed acceptable if they were within 10% of the target concentrations; for the 12.2 μg per ml concentration, due to analytical limitations, 20%of the target concentration was considered acceptable. Dose certification analyses were conducted on all glycidamide drinking water solutions and each met the indicated specifications.

For the 3-month study, drinking water solutions were prepared at 2 to 3 week intervals beginning on 28 July 2004 and ending on 21 October 2004. The target concentrations were 12.2, 30.6, 61.2, 122, and 306 μg glycidamide per ml corresponding to 0.14, 0.35, 0.70, 1.41, and 3.52 mM glycidamide. Concentrations were deemed acceptable if they were within 10% of the target concentrations; for the 12.2 μg per ml concentration, 20% of the target concentration was considered acceptable. Solutions prepared on 28 July 2004 were found to contain 74.0% to 84.3% of the desired concentrations; the 30.6 and 306 μg per ml solutions prepared on 16 August 2004 were found to contain 72.9% to 74.8% of the desired concentrations. The rats received these low doses of glycidamide for approximately 3 weeks. The low concentrations of glycidamide were traced to the use of a stainless-steel mixing vessel. Subsequent mixes were conducted with a polypropylene vessel, which eliminated the problem of low recoveries. The 61.2 μg per ml drinking water solution prepared on 10 September 2004 was found to contain

119% of the desired concentration; two additional analyses indicated a value of 113%. All the other glycidamide drinking water solutions met the indicated specifications.

For the 2-year study, glycidamide drinking water solutions for treating the animals were prepared weekly, beginning on 24 May 2005 and ending on 14 August 2007. The target concentrations were 7.65, 15.3, 30.6, and 61.2 μg glycidamide per ml corresponding to 0.0875, 0.175, 0.35, and 0.70 mM glycidamide. Concentrations were deemed acceptable if they were within 10% of the target concentrations; for the 7.65 μg per ml concentration, 20% of the target concentration was considered acceptable. Dose certification analyses were conducted at approximately bi-monthly intervals (Table F-4). Each of the assayed glycidamide drinking water solutions met the indicated specifications. Glycidamide was not detected in the control drinking water solutions (limit of quantitation was 1.5 μg per ml).

Two-week Studies

F344/N Nctr rats and B6C3F1/Nctr (C57BL/6N × C3H/HeN MTV$^-$) mice were obtained from the NCTR breeding colony at three weeks of age. The animals were tail-tattooed for identification, weight-ranked, and randomly loaded on the MultiGen Support System. In addition, mice were ear-clipped for identification. Treatment was initiated when the animals were 4 to 5 weeks of age. On the first day of dosing, female rats weighed between 37.3 g and 98.2 g, male rats weighed between 63.1 g and 100.4 g, female mice weighed between 13.6 g and 16.4 g, and male mice weighed between 16.3 g and 20.8 g.

Groups of four F344/N Nctr rats per sex and four B6C3F1/Nctr mice per sex were dosed with 0.0, 0.14, 0.35, 0.70, 1.41, 3.52, or 7.03 mM glycidamide in the drinking water (0, 12.2, 30.6, 61.2, 122, 306, or 612 ppm glycidamide). The animals were treated for 14 days and were monitored twice daily, in the morning and afternoon. The rats were housed two of the same sex per cage and mice were housed four of the same sex per cage. Purina 5LG6 diet (also referred to as NIH-31 IR) was selected for the study because it has a very low acrylamide content (< 50 ppb) compared to other commercial formulations[57]. Irradiated Purina 5LG6 meal and Millipore-filtered tap water were provided ad libitum. Feed was subjected to routine chemical analyses. The acrylamide content of the 5LG6 diet was determined to be 28 ± 25 ng per ml (n = 9; Table H-4); acrylamide was not detected in the control drinking water solutions (limit of quantitation 2 μg/ml). The animal rooms were maintained on a 12-hour light-dark cycle, with 10 to 15 air changes per hour. Environmental controls were set to maintain the temperature at 22 ± 4°C, with a relative humidity of 40% to 70%. Body weights were recorded on dose days 1, 7, and 14. Food and water consumption was measured weekly.

On the afternoon of dose day 14, the animals were delivered to the necropsy holding area. They continued to receive dosed water. On dose day 15, all animals were weighed (designated as necropsy body weight) and euthanized by exposure to carbon dioxide. Under the supervision of a pathologist, a gross examination was performed on all animals. Gross examination data were recorded with the Individual Animal Necropsy Recording system. The livers and brains were dissected and weighed. Gross lesions and the following organs were processed for microscopic examination: brain (cerebrum, cerebellum, and brain stem), Harderian glands, heart, liver, lungs, peripheral nerve (sciatic), ovaries, thyroid gland, parathyroid gland, skin, mammary glands, spinal cord (thoracic, lumbar, and cervical), forestomach, glandular stomach, and testes. The pathology data were recorded in Micropath.

Three-month Studies

F344/N Nctr rats and B6C3F1/Nctr (C57BL/6N × C3H/HeN MTV⁻) mice were obtained from the NCTR breeding colony at three weeks of age. Rats were tail-tattooed and mice were ear-clipped for identification. Mice were also tail-tattooed at 8 to 12 weeks of age. The animals were weight-ranked, and randomly loaded on the MultiGen Support System. Treatment was initiated when the rats were 4 to 5 weeks of age and the mice were 5 to 6 weeks of age. On the first day of dosing, female rats weighed between 84.7 g and 117.0 g, male rats weighed between 106.3 g and 139.7 g, female mice weighed between 11.8 g and 16.5 g and male mice weighed between 13.6 g and 20.3 g.

The rats were housed two of the same sex per cage and mice were housed four of the same sex per cage in polycarbonate cages with hardwood chips bedding. Irradiated Purina 5L6G meal and Millipore-filtered tap water were provided ad libitum. Feed and water were subjected to routine microbiological and chemical analyses. The animal rooms were maintained on a 12-hour light-dark cycle, with 10 to 15 air changes per hour. Environmental controls were set to maintain the temperature at 22 ± 4°C, with a relative humidity of 40% to 70%.

Each dose group consisted of eight animals per sex. The dosage groups were 0.0, 0.14, 0.35, 0.70, 1.41, or 3.52 mM glycidamide in the drinking water (0, 12.2, 30.6, 61.2, 122, or 306 ppm glycidamide). The animals were treated for 3 months and were monitored twice daily, in the morning and afternoon. Body weights, food consumption, and water consumption were measured weekly.

On the afternoon before the scheduled terminal sacrifice, the animals were delivered to the necropsy holding area. They continued to receive dosed water. On the following day, all animals were weighed (designated as necropsy body weight), and euthanized by exposure to carbon dioxide. Under the supervision of a pathologist, a gross examination was performed on all animals. Gross examination data were recorded with the Individual Animal Necropsy Recording system. The livers and brains were dissected and weighed. Gross lesions and the following organs were processed for microscopic examination: adrenal glands, bone marrow (femur), brain (cerebrum, cerebellum, and brain stem), clitoral glands, epididymides, esophagus, eyes, Harderian glands, heart (aorta), intestine (large: cecum, colon, and rectum), intestine (small: duodenum, jejunum, and ileum), kidneys, liver, lungs/bronchi, lymph nodes (mesenteric and mandibular), muscle (thigh), nerve (sciatic), nose, ovaries, pancreas, parathyroid glands, pituitary glands, preputial glands, prostate, salivary glands, seminal vesicles, skin (mammary glands), spinal cord (thoracic, lumbar, and cervical), spleen, stomach (forestomach and glandular), testes, thymus, thyroid glands, tongue, trachea, urinary bladder, and uterus. The pathology data were recorded in the Laboratory Data Acquisition System (LDAS).

Two-year Studies

Study Design

Each dose group consisted of 48 animals per sex per species. The dosage groups were 0, 0.0875, 0.175, 0.35, and 0.70 mM glycidamide in the drinking water (0, 7.65, 15.3, 30.6, and 61.2 ppm glycidamide). The animals were treated for 2 years and were monitored twice daily, in the

morning and afternoon. Body weights, food consumption, and water consumption were measured weekly.

Source and Specification of Animals

Male and female F344/N Nctr rats were obtained from the NCTR breeding colony at 3 weeks of age, tail-tattooed for identification, weight-ranked, and randomly loaded on the MultiGen Support System. The animals were loaded to the study in twelve balanced replicates. Treatment was initiated when the rats were 4 to 5 weeks of age. On the first day of dosing, the female rats weighed between 50.9 g and 110.8 g; the male rats weighed between 65.5 g and 123.6 g.

Male and female B6C3F1/Nctr (C57BL/6N × C3H/HeN MTV$^-$) mice were obtained from the NCTR breeding colony at 3 weeks of age, ear-clipped for identification (at 8 to 12 weeks of age, their tails were also tattooed to provide additional identification), weight ranked, and randomly loaded on the MultiGen Support System. The animals were loaded to the study in twelve balanced replicates. Treatment was initiated when the mice were 5 to 6 weeks of age. On the first day of dosing, the female mice weighed between 11.5 g and 18.9 g; the male mice weighed between 13.9 g and 21.3 g.

Animal Maintenance

All animal experimental procedures were performed in accordance with an animal study protocol approved by the Institutional Animal Care and Use Committee at the NCTR.

The rats were housed two of the same sex per cage in polycarbonate cages with hardwood chip bedding. The mice were housed four of the same sex per cage in polycarbonate cages with hardwood chip bedding and micro-isolator tops. Microbiological surveillance of sentinel rats and mice was conducted on a routine basis (Appendix I).

Irradiated Purina 5LG6 meal and Millipore-filtered tap water were provided ad libitum. Feed was subjected to routine chemical analyses; water underwent routine microbiological surveillance.

The animal rooms were maintained on a 12-hour light-dark cycle, with 10 to 15 air changes per hour. Environmental controls were set to maintain the temperature at 22 ± 4°C, with a relative humidity of 40% to 70%. Microbiological surveillance of the animal rooms was conducted on a routine basis.

Clinical Examinations and Pathology

On the afternoon before the scheduled terminal sacrifice, the animals were delivered to the necropsy holding area. They continued to receive the dosed water. On the following day, all animals were weighed (designated as necropsy body weight) and then euthanized by exposure to carbon dioxide. Under the supervision of a pathologist, complete necropsies were performed on all terminal sacrifice animals. Complete necropsies were also performed on all animals that died naturally or that were submitted moribund prior to the scheduled terminal sacrifice. The protocol-designated tissues (see below) were examined grossly, removed, and preserved in 10% neutral buffered formalin, except the eyes and testes, which were placed in modified Davidson's fixative. Gross findings were recorded in the automated Gross Pathology System. The protocol-designated tissues were trimmed, processed, and embedded in Formula R® infiltrating medium, sectioned at approximately 5 microns, and stained with hematoxylin and eosin for microscopic

evaluation. In a few cases, special staining procedures were applied to selected lesions to aid in characterizing the pathology changes. The protocol-designated tissues were: brain (cerebrum, cerebellum, and brain stem), Harderian glands, heart, liver, lungs, pancreas, peripheral nerve (sciatic), ovaries, thyroid gland, parathyroid gland, skin, mammary glands, spinal cord (thoracic, lumbar, and cervical), forestomach, glandular stomach, duodenum, ileum, jejunum, cecum, colon, rectum, testes, kidneys, urinary bladder, spleen, prostate, trachea, esophagus, uterus, eye, aorta, nose, pituitary, preputial/clitoral glands, epididymis, lymph nodes (mesenteric and mandibular), seminal vesicles, thymus, salivary glands, bone (femur), and adrenal glands.

Upon completion of the microscopic evaluations, the pathology data were entered into the LDAS. The slides, paraffin blocks, and residual wet tissues were sent to the Block and Slide Laboratory for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment group. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. A quality assessment pathologist evaluated slides of all proliferative lesions and target organs. Differences of opinion were reconciled between the study pathologist and the quality assessment pathologist.

Histopathology slides containing the diagnoses made by the study pathologist and quality assessment pathologist were reviewed by a Pathology Working Group (PWG). The PWG consisted of the quality assessment pathologist, the study pathologist, and other pathologists experienced in rodent toxicologic pathology. The quality assessment pathologist served as the coordinator. Representative histopathology slides containing examples of lesions related to glycidamide administration, examples of disagreements in diagnoses between the study pathologist and quality assessment pathologist, and lesions of general interest were presented by the Coordinator to the PWG for review. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. Final diagnoses for reviewed lesions represent a consensus between the study pathologist, reviewing pathologist, and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman[58] and Boorman et al.[59]. For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell et al.[60].

Acrylamide is neurotoxic in experimental animals (reviewed in NTP[4]), and the possibility exists that glycidamide is likewise. In view of this possibility, an additional Pathology Quality Assessment Review was conducted on sections of brain, spinal cord, and peripheral nerve. This additional review was conducted by pathologists from an independent laboratory who had special expertise in neuropathology. During this review, all changes in the nervous system were documented, regardless of their severity. Based upon these very stringent criteria, additional lesions in the nervous system were detected. Subsequently, a special PWG, consisting of six experienced pathologists, was convened to evaluate the results. The special PWG utilized stringent criteria similar to those used in the special neuropathology quality assessment in making their evaluations and recommended that all of the lesions, regardless of their severity, be added to the pathology results for the study. This recommendation was adopted.

The experimental design and materials and methods for the 2-week, 3-month, and 2-year drinking waters studies of glycidamide are summarized in Table 2.

Table 2. Experimental Design and Materials and Methods in the Drinking Water Studies of Glycidamide

Two-week Studies	Three-month Studies	Two-year Studies
Study Laboratory		
U.S. FDA National Center for Toxicological Research (NCTR, Jefferson, AR)	Same as 2-week studies	Same as 2-week studies
Strain and Species		
Rats: F344/N Nctr Mice: B6C3F1/Nctr (C57BL/6N × C3H/HeN MTV−)	Same as 2-week studies	Same as 2-week studies
Animal Source		
NCTR breeding colony	Same as 2-week studies	Same as 2-week studies
Time Held Before Studies		
1 to 2 weeks	1 to 2 weeks	2 to 3 weeks
Average Age When Studies Began		
4 to 5 weeks	4 to 5 weeks	5 to 6 weeks
Date of First Exposure		
Rats: April 12, 2004	Rats: August 4/5, 2004	Rats: May 30/31, 2005; June 6/7, 13/14, 20/21, and 27/28, 2005; July 4/5, 11/12, 18/19, and 25/26, 2005; and August 1/2, 8/9, and 15, 2005
Mice: April 13, 2004	Mice: August 2/3, 2004	Mice: June 2, 9, 16, 23, and 30, 2005; July 7, 14, 21, and 28, 2005; and August 4, 11, and 18, 2005
Duration of Exposure		
2 weeks	13 weeks	104 weeks
Date of Last Exposure		
Rats: April 27, 2004	Rats: November 4/5, 2004	Rats: June 6, 13, 20, and 27, 2007; July 5, 11, 18, 25, and 31, 2007; and August 8, 15, and 22, 2007
Mice: April 28, 2004	Mice: November 2/3, 2004	Mice: June 5, 12, 19, and 26, 2007; July 3, 10, 17, 24, and 31, 2007; and August 7, 14, and 21, 2007
Necropsy Dates		
Rats: April 27, 2004	Rats: November 4/5, 2004	Rats: June 6, 13, 20, and 27, 2007; July 5, 11, 18, 25, and 31, 2007; and August 8, 15, and 22, 2007
Mice: April 28, 2004	Mice: November 2/3, 2004	Mice: June 5, 12, 19, and 26, 2007; July 3, 10, 17, 24, and 31, 2007; and August 7, 14, and 21, 2007
Average Age at Necropsy		
6 to 7 weeks	17 to 18 weeks	2 years

Two-week Studies	Three-month Studies	Two-year Studies
Size of Study Groups		
4 males and 4 females	8 males and 8 females	48 males and 48 females
Method of Distribution		
Animals were distributed randomly into groups of approximately equal initial body weights.	Same as 2-week studies	Same as 2-week studies
Animals per Cage		
Rats: 2 same sex	Same as 2-week studies	Same as 2-week studies
Mice: 4 same sex		
Method of Animal Identification		
Rats: Tail tattoo	Same as 2-week studies	Same as 2-week studies
Mice: Ear clip and tail tattoo		
Diet		
Irradiated Purina 5LG6 meal feed (also designated NIH-31 IR), available ad libitum	Same as 2-week studies	Same as 2-week studies
Water		
Millipore-filtered tap water, available ad libitum	Same as 2-week studies	Same as 2-week studies
Cages		
Polycarbonate cages (Lab Products, Inc., Seaford, DE and Allentown Caging and Equipment, Allentown, NJ), changed twice weekly (rats) or once weekly (mice)	Same as 2-week studies	Same as 2-week studies
Bedding		
Autoclaved hardwood chip bedding (Northeastern Products Corp., Caspian, MI), changed twice weekly (rats) or once weekly (mice)	Same as 2-week studies	Same as 2-week studies
Cage Filters		
Spunbonded polyester (Lab Products, Inc., Seaford, DE and Allentown Caging and Equipment, Allentown, NJ), changed every 2 weeks	Same as 2-week studies	Same as 2-week studies
Racks		
Stainless steel (Research Equipment Co., Bryan, TX), changed every 3 weeks	Same as 2-week studies	Same as 2-week studies

Two-week Studies	Three-month Studies	Two-year Studies
Animal Room/Chamber Environment		
Temperature: 22° ± 4°C Relative humidity: 40% to 70% Room fluorescent light: 12 hours/day Room air changes: 10 to 15/hour	Same as 2-week studies	Same as 2-week studies
Exposure Concentrations		
0.0, 0.14, 0.35, 0.70, 1.41, 3.52, and 7.03 mM glycidamide (0, 12.2, 30.6, 61.2, 122, 306, and 612 ppm glycidamide)	0.0, 0.14, 0.35, 0.70, 1.41, and 3.52 mM glycidamide (0, 12.2, 30.6, 61.2, 122, and 306 ppm glycidamide)	0.0, 0.0875, 0.175, 0.35, and 0.70 mM glycidamide (0, 7.65, 15.3, 30.6, and 61.2 ppm glycidamide)
Type and Frequency of Observation		
Observed twice daily; animals were weighed on dose days 1, 7, and 14; and food and water consumption measured weekly	Observed twice daily; animals were weighed weekly; and food and water consumption were measured weekly	Same as 3-month studies
Method of Sacrifice		
Carbon dioxide asphyxiation	Same as 2-week studies	Same as 2-week studies
Necropsy		
Necropsies were performed on all animals. Organs weighed were liver and brain. Processing for microscopic examination was performed on gross lesions, brain (cerebrum, cerebellum, and brain stem), Harderian glands, heart, liver, lungs, pancreas, peripheral nerve (sciatic), ovaries, thyroid gland, parathyroid gland, skin, mammary glands, spinal cord (thoracic, lumbar, and cervical), forestomach, glandular stomach, and testes.	Necropsies were performed on all animals. Organs weighed were liver and brain. Processing for microscopic examination was performed on gross lesions, brain (cerebrum, cerebellum, and brain stem), Harderian glands, heart, liver, lungs, pancreas, peripheral nerve (sciatic), ovaries, thyroid gland, parathyroid gland, skin, mammary glands, spinal cord (thoracic, lumbar, and cervical), forestomach, glandular stomach, and testes.	Necropsies were performed on all animals. Processing for microscopic examination was performed on gross lesions, brain (cerebrum, cerebellum, and brain stem), Harderian glands, heart, liver, lungs, pancreas, peripheral nerve (sciatic), ovaries, thyroid gland, parathyroid gland, skin, mammary glands, spinal cord (thoracic, lumbar, and cervical), forestomach, glandular stomach, duodenum, ileum, jejunum, cecum, colon, testes, kidneys, urinary bladder, spleen, prostate, trachea, esophagus, uterus, eye, aorta, nose, pituitary, preputial/clitoral gland, epididymis, lymph nodes (mesenteric and mandibular), seminal vesicles, thymus, salivary glands, bone (femur), and adrenal glands.

Statistical Methods

Survival Analyses

Mean and median survival times and plots of rodent survival functions were obtained using Kaplan-Meier estimation[61]. Cox proportional hazards regression analyses[62] were conducted to compare the hazard function of each dose group to that of the control group and to test for a linear trend between the hazard and glycidamide dose. The hazard for each dose group was defined as a function of both glycidamide dose and time on study, measured in weeks.

Differences in survival at each dose between B6C3F1/Nctr mice given acrylamide in the drinking water for 2 years[4] and B6C3F1/Nctr mice given glycidamide in the drinking water in the current study were also investigated. Homogeneity of survival curves was tested at each dose level between mice administered acrylamide and those administered glycidamide. A similar analysis was conducted for F344/N Nctr rats given acrylamide in the drinking water for 2 years[4] and F344/N Nctr rats given glycidamide in the drinking water in the current study.

Body Weight Analyses

The effect of glycidamide dose on body weight was investigated using a sex-stratified, repeated measures, mixed models analysis of variance (ANOVA), with dose and week main effects and a dose × week interaction effect. Within-group correlations were modeled using a heterogeneous first order autoregressive (ARH(1)) covariance structure that allows for (1) increasing variability in the animal's weight over time and (2) body weights being correlated at adjacent time points to a greater extent than at distant time points. Least squares estimates of mean body weight were obtained for each dose group from weeks 4 to 104 in 4 week intervals. Pairwise comparisons of dose group to control group (0.0 mM glycidamide) body weight means were performed to determine if there was a difference between the control and the respective dose group means. Dunnett's adjustment[63] was used to correct for multiple pairwise comparisons to controls. Trend tests were conducted to determine if body weight means decreased or increased with increasing dose.

The difference in mean body weight at each dose between B6C3F1/Nctr mice given acrylamide in the drinking water for 2 years[4] and B6C3F1/Nctr mice given glycidamide in the drinking water in the current study was investigated using a sex-stratified, repeated measures, mixed models, three-way ANOVA with main effects of compound, dose, week, and all higher-order interaction effects. Within-group correlations were modeled using a heterogeneous first order autoregressive (ARH(1)) covariance structure, which allows for (1) differences in weight variability over time, and (2) body weights being correlated more at adjacent time points than at distant time points. Least squares estimates of mean body weight were obtained for each compound and dose group at 4 week intervals. A similar analysis was conducted for F344/N Nctr rats given acrylamide in the drinking water for 2 years[4] and F344/N Nctr rats given glycidamide in the drinking water in the current study.

Water and Food Consumption Analyses

The effect of glycidamide dose on food and water consumption was determined on a cage basis. For each cage and for each consumption period, food and water consumption were calculated by subtracting the container weight at the end of the period from the container weight at the

beginning of the period. Consumption periods were grouped into 4 week study periods based on the observation date. The sum of the food and water consumption within the study period was then divided by the number of animal-days to obtain the food and water consumption per day for each study period for each animal. With study periods defined as such the effect of glycidamide dose on food and water consumption was analyzed using a sex-stratified, repeated measures, mixed models ANOVA with dose and study period main effects and a dose by study period interaction effect. Within-group correlations were modeled using a heterogeneous first order autoregressive (ARH(1)) covariance structure that allows for (1) differences over time in the variability of the amount of food and water consumed, and (2) the amount of consumed food and water being correlated to a greater extent at adjacent time points than at distant time points. Least squares estimates of the mean amount of food and water consumed were obtained for each dose group at four week intervals. Pairwise comparisons of the amount of food and water consumed by the dose group to that of the control group were performed to determine if there was a difference between the control and the respective dose group means. Dunnett's adjustment[63] was used to correct for multiple pairwise comparisons to controls. Trend tests were conducted to determine if the mean amount of food and water consumed decreased or increased with increasing dose.

The difference in mean food and water consumption at each dose between B6C3F1/Nctr mice given acrylamide in the drinking water for 2 years[4] and B6C3F1/Nctr mice given glycidamide in the drinking water in the current study was also examined. For each cage and for each consumption period food and water consumption was calculated by subtracting the container weight at the end of the period from the container weight at the beginning of the period. Consumption periods were grouped into 4-week study periods based on the observation date. The sum of the food (or water) consumptions within the study period was then divided by the number of animal-days to obtain the food (or water) consumption per day for each study period for each animal. Differences in mean food and water consumption were then tested using a sex-stratified, repeated measures, mixed models, three-way ANOVA with main effects of compound, dose, week, and all higher-order interaction effects. Within-group correlations were modeled using a heterogeneous first order autoregressive (ARH(1)) covariance structure, which allows for (1) differences over time in the variability of the amount of food and water consumed, and (2) the amount of consumed food and water being correlated more at adjacent time points than at distant time points. Least squares estimates of mean amount of food and water consumed were obtained for each compound and dose group at 4 week intervals. A similar analysis was conducted for F344/N Nctr rats given acrylamide in the drinking water for 2 years[4] and F344/N Nctr rats given glycidamide in the drinking water in the current study.

Water consumption and body weight data were used to determine glycidamide exposure. For each consumption period, the amount of water consumed per cage was calculated by subtracting the container weight at the end of the period from the container weight at the beginning of the period. The amount of body weight-days for a cage in a consumption period was computed by first multiplying, for each animal in a cage, the body weight of each mouse or rat by the number of days that mouse or rat was on study in that consumption period, and then summing these products over all the mice or rats in the cage. For each cage of mice or rats, the amount of compound consumed in milligrams per kilogram of animal weight per day was then calculated by dividing the amount of water consumed per cage by the calculated body weight-days per cage, and then converting this quantity to milligrams using the dose concentration and the

molecular weight of glycidamide (87.08 g per mol). Consumption periods were then grouped into 1-week study periods based on the observation date.

Differences in mean compound consumption at each dose between B6C3F1/Nctr mice given acrylamide in the drinking water for 2 years[4] and B6C3F1/Nctr mice given glycidamide in the drinking water in the current study were also investigated using a sex and dose-stratified fixed effects analysis of variance (ANOVA) with main effects of compound and study period and a compound by study period interaction effect. Comparisons of the amount of compound consumed by the acrylamide-treated mice to the corresponding glycidamide-treated mice were conducted to determine if there was a difference in least squares mean compound consumption between mice treated with acrylamide and those treated with glycidamide. A similar analysis was conducted for F344/N Nctr rats given acrylamide in the drinking water for 2 years[4] and F344/N Nctr rats given glycidamide in the drinking water in the current study.

Pathology Data Analyses

The continuity-corrected Poly-3 test[64], as modified by Bieler and Williams[65], was used to assess prevalence of neoplasms and nonneoplastic lesions. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. 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 one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice 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 3rd power. This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time[64]. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions.

Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall exposure-related trend. Continuity-corrected Poly-3 tests[66] were used in the analysis of neoplasm incidence; the reported P values are one sided. Positive trends are reported with right-tailed P values. Negative trends are reported with left-tailed P values, with the letter N added to indicate a lower incidence as exposure increases. P values <0.05 were considered significant.

Differences in tumor incidences between B6C3F1/Nctr mice given acrylamide in the drinking water for 2 years[4] and B6C3F1/Nctr mice given glycidamide in the drinking water in the current study were also investigated. Logistic regression using Poly-3 weights was used to model the dose response of acrylamide and glycidamide and determine if the slopes of their regression lines were equal. The model included a continuous dose term and a term for the interaction of dose with compound. Equality of slopes between the regression lines was assessed by testing the statistical significance of the interaction effect. Logistic regression using Poly-3 weights and conditioning on each dose level was used to compare tumor incidence between the two compounds at each dose. A similar analysis was conducted for F344/N Nctr rats given acrylamide in the drinking water for 2 years[4] and F344/N Nctr rats given glycidamide in the drinking water in the current study.

Quality Assurance

This study was conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations[67]. The Quality Assurance Unit at the NCTR performed audits and inspections of the protocols, procedures, data, and reports throughout the course of the study. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this technical report were conducted. Audit procedures and findings are on file at the NCTR. The audit findings were reviewed and assessed by the NCTR staff, and all comments were resolved or otherwise addressed either before or during the preparation of the technical report.

Raw data sheets from the study are archived by the NCTR's record management unit. Histopathology samples collected during the course of the study are stored in the archives of Toxicologic Pathology Associates at the NCTR. Backup computer data are maintained by the computer staff at the NCTR. All records and samples are stored in accordance with Food and Drug Administration Good Laboratory Practice Regulations.

Results

Rats

Two-week Study

One female rat given 7.03 mM glycidamide in the drinking water died after 12 days of treatment. Hind limb paresis was observed on day 14 in four of four male rats and one of four female rats administered 7.03 mM glycidamide in the drinking water (Table 3). Paresis was not observed in any other treatment groups. There were no other significant in-life observations in any of the other treatment groups.

Table 3. Incidence of Observations and Nonneoplastic Lesions in Rats in the Two-week Glycidamide Studies[a]

	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM	7.03 mM
Males							
Animals initially in study	4	4	4	4	4	4	4
Hind limb							
Paresis	0	0	0	0	0	0	4
Urinary bladder							
Dilatation	0	0	0	0	0	0	3 (2.6)
Testes							
Seminiferous tubule degeneration	0	0	0	0	0	0	4 (2.5)
Females							
Animals initially in study	4	4	4	4	4	4	4
Hind limb							
Paresis	0	0	0	0	0	0	1
Urinary bladder							
Dilatation	0	0	0	0	0	0	1 (3.0)

[a]Data are reported as the number of lesions per number of rats (4) examined microscopically. The average severity is given in parentheses. **Severity was scored as: 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.**

Male and female rats administered 7.03 mM glycidamide and male rats given 3.52 mM glycidamide in the drinking water for 14 days had significantly decreased body weights as compared to controls (Table 4). Water consumption generally paralleled body weight changes, with groups given the highest dose of glycidamide typically having the lowest consumption of drinking water (Table 4). The same trend occurred with food consumption (Table 4).

Male rats administered 0.14, 0.35, 0.70, 1.41, 3.52, or 7.03 mM glycidamide in the drinking water consumed approximately 1.8, 4.8, 9.9, 20.5, 49.7, or 89.7 mg glycidamide per kg body weight per day; the comparable values for female rats were 2.3, 5.3, 10.6, 24.2, 47.0, or 138 mg glycidamide per kg body weight per day.

Necropsy body weights, liver weights, and liver to brain weight ratios were decreased in all rats administered 7.03 mM glycidamide in the drinking water for 14 days (Table E-1). The necropsy body weights were decreased in male rats receiving 3.52 mM glycidamide.

Dilatation of the urinary bladder was observed grossly in three of four male rats given 7.03 mM glycidamide in the drinking water, in one of four females given 7.03 mM glycidamide in the drinking water (Table 3). When dilatation was observed grossly, the lesion was examined microscopically, confirming the presence of the lesion with a mild-to-moderate average severity.

Most of the rats having dilatation of the urinary bladder had displayed hind limb paresis. This correlation suggested that the dilatation of the urinary bladders in these rats may have been due to impairment of neurological function rather than to a direct toxic effect on the urinary bladder; however, microscopic examination of three levels of brain, three levels of spinal cord, and sciatic nerves of all of these animals failed to reveal any morphologic changes in nervous tissue that could be attributed to glycidamide administration.

A mild-to-moderate average severity for degeneration of the germinal epithelium in the seminiferous tubules of the testes was noted microscopically in all male rats given 7.03 mM glycidamide in the drinking water (Table 3). The lesion was characterized by decreased numbers of germinal cells and the presence of multinucleated spermatids in the lumens of seminiferous tubules.

Exposure Concentration Selection Rationale: The selection of doses for the 3-month glycidamide drinking water study was based upon the effects observed in the 2-week studies of glycidamide (this study) and acrylamide[4], which were conducted simultaneously. Both 7.03 mM glycidamide and 7.03 mM acrylamide in drinking water resulted in hind limb paresis and decreased body weight. Since one of the goals of this study was to compare acrylamide with glycidamide, a high dose of 3.52 mM glycidamide (306 ppm glycidamide) was selected for the 3-month drinking water study, with the remaining doses being 0, 0.14, 0.35, 0.70, and 1.41 mM glycidamide (0, 12.2, 30.6, 61.2, and 122 ppm glycidamide). These doses were identical to those used in the 3-month drinking water study with acrylamide[4].

Table 4. Survival, Body Weights, Food Consumption, and Water Consumption of Rats in the Two-week Drinking Water Study of Glycidamide

Treatment	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Mean Food Consumption[c]		Mean Water Consumption[c]	
		Day 1	Day 7	Day 14		Week 1	Week 2	Week 1	Week 2
Drinking Water									
Male									
0.0 mM	4/4	86.7 ± 5.1	115.7 ± 7.2	143.6 ± 6.9		12.9 (100)	15.6 (100)	18.2[d] (100)	20.3 (100)
0.14 mM	4/4	82.6 ± 10.0	117.6 ± 8.8	148.1 ± 8.5	103	14.3 (111)	15.3 (98)	18.6 (102)	20.7 (102)
0.35 mM	4/4	80.6 ± 6.5	109.4 ± 5.2	136.5 ± 5.4	95	14.1 (109)	15.1 (97)	17.7 (97)	20.9 (103)
0.70 mM	4/4	82.3 ± 7.2	118.7 ± 7.2	147.8 ± 7.3	103	14.7 (114)	15.9 (102)	20.6 (113)	22.3 (110)
1.41 mM	4/4	82.7 ± 3.6	116.0 ± 4.1	144.0 ± 4.7	100	14.0 (109)	15.9 (102)	20.7[d] (114)	22.3 (110)
3.52 mM	4/4	81.1 ± 3.3	100.4 ± 1.5	120.6 ± 1.9*	84	11.8 (91)	13.7 (88)	17.4 (96)	18.2 (90)
7.03 mM	4/4	80.6 ± 0.8	81.9 ± 0.4*	86.4 ± 1.2*	60	11.3 (88)	12.0 (77)	12.9 (71)	11.7 (58)
Female									
0.0 mM	4/4	61.2 ± 8.0	85.5 ± 6.5	104.8 ± 4.1		12.9 (100)	12.6 (100)	13.1 (100)	18.1 (100)
0.14 mM	4/4	60.2 ± 14.2	84.5 ± 12.5	105.0 ± 9.8	100	12.8 (99)	13.0 (103)	15.7 (120)	19.9 (110)
0.35 mM	4/4	62.8 ± 13.6	85.6 ± 11.7	106.8 ± 10.2	102	11.3 (88)	12.7 (101)	16.3[d] (124)	16.8 (93)
0.70 mM	4/4	64.5 ± 8.0	88.7 ± 6.5	108.7 ± 4.5	104	11.4 (88)	13.0 (103)	16.4 (125)	17.8 (98)
1.41 mM	4/4	63.4 ± 6.1	89.0 ± 3.6	106.1 ± 3.3	101	12.1 (94)	12.9 (102)	19.3 (147)	18.8 (104)
3.52 mM	4/4	63.1 ± 3.7	76.2 ± 2.8	91.3 ± 1.9	87	9.1 (71)	10.7 (85)	10.5 (80)	15.4 (85)
7.03 mM	3/4	65.3 ± 11.4	62.6 ± 7.4	60.5 ± 10.2*	58	7.1 (55)	6.6 (52)	19.2 (147)	8.7 (48)

[a]Number of animals surviving at 14 days/number initially in group.
[b]Weights are given as mean ± standard error. An asterisk (*) denotes those that are significantly different ($p < 0.05$) from controls.
[c]Food and water consumption are expressed as grams per animal per day and were measured on a per cage basis and presented as mean of two cages and, in parentheses, the percentage of the respective control. Statistical analyses were not conducted on food and water consumption because there were only two cages per treatment group.
[d]Data based upon one cage only.

Three-month Study

All animals survived to the end of the 3-month experiment. In rats administered 3.52 mM glycidamide in the drinking water, a 100% incidence of hind limb paresis was observed after approximately 5 weeks of treatment. The paresis was not of sufficient severity to necessitate removing the animals from the study (Table 5).

Table 5. Incidence of Observations and Nonneoplastic Lesions in Rats in the Three-month Drinking Water Study of Glycidamide[a]

	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM
Males						
Animals initially in study	8	8	8	8	8	8
Hind limb						
Paresis	0	0	0	0	0	8
Peripheral nerve						
Axon degeneration	0	–[b]	–	–	–	0
Schwann cell degeneration	0	–	–	–	–	0
Spinal cord						
Lumbar axon degeneration	0	–	–	–	–	2 (1.0)
Urinary bladder						
Dilatation	0	–	–	–	–	2 (2.5)
Spleen						
Congestion	0	–	–	–	–	1 (2.0)
Pigmentation	0	–	–	–	–	0
Testes						
Germinal epithelium degeneration	0	2 (1.0)	3 (1.0)	8 (1.9)	8 (2.8)	8 (3.9)
Epididymis						
Exfoliated germ cell	0	0	3 (1.0)	8 (1.9)	8 (3.0)	8 (4.0)
Hypospermia	0	0	0	6 (1.5)	8 (2.9)	8 (4.0)
Females						
Animals initially in study	8	8	8	8	8	8
Hind limb						
Paresis	0	0	0	0	0	8
Peripheral nerve						
Axon degeneration	0	–	–	–	–	1 (1.0)
Schwann cell degeneration	0	–	–	–	–	1 (1.0)
Spinal cord						
Lumbar axon degeneration	0	–	–	–	–	3 (1.3)
Urinary bladder						
Dilatation	0	–	–	–	–	0
Spleen						
Congestion	0	–	–	–	–	0
Pigmentation	0	–	–	–	–	0

[a]Data are reported as the number of lesions per number of rats (8) examined microscopically. The average severity is given in parentheses. **Severity was scored as: 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.**
[b]Not examined. If paresis was observed, then a microscopic examination of the peripheral nerve and spinal cord was conducted.

There were significant dose-related effects in body weight in both male and female rats exposed to glycidamide in the drinking water (Table 6 and Figure 5). Pairwise comparisons indicated significant decreases in body weight gain in both sexes over the 3-month treatment period in rats administered 1.41 or 3.52 mM glycidamide. Mean body weights in the 3.52 mM glycidamide group were depressed by >10% after two (females) to three (males) weeks of dosing and at the end of the 3-month period, the rats weighed 78% of their respective control groups (Table 6). Mean body weights in the 1.41 mM glycidamide group were depressed by >10% after six (males) to seven (females) weeks of dosing and at the end of the 3-month, the rats weighed 86% (males) to 88% (females) of their respective control groups (Table 6).

Table 6. Survival and Body Weights of Rats in the Three-month Drinking Water Studies of Glycidamide

Treatment	Survival[a]	Mean Body Weight[b] (g)		Final Weight Relative to Controls (%)
		Week 0	Week 14	
Male				
0.0 mM	8/8	121.6 ± 2.2	340.6 ± 2.2	
0.14 mM	8/8	120.1 ± 2.2	329.6 ± 2.2	97
0.35 mM	8/8	123.2 ± 2.2	340.3 ± 2.2	100
0.70 mM	8/8	121.4 ± 2.2	325.7 ± 2.2	96
1.41 mM	8/8	122.7 ± 2.2	292.9 ± 2.2*	86
3.52 mM	8/8	119.7 ± 2.2	264.8 ± 2.2*	78
Female				
0.0 mM	8/8	104.8 ± 1.2	202.6 ± 1.2	
0.14 mM	8/8	101.1 ± 1.2	198.5 ± 1.2	98
0.35 mM	8/8	98.9 ± 1.2	191.7 ± 1.2	95
0.70 mM	8/8	101.9 ± 1.2	195.4 ± 1.2	97
1.41 mM	8/8	98.5 ± 1.2	177.7 ± 1.2*	88
3.52 mM	8/8	100.3 ± 1.3	157.4 ± 1.2*	78

[a]Number of animals surviving until study termination/number of animals initially in group.
[b]Weights are given as LS means ± population standard error of the mean. An asterisk (*) denotes those that are significantly different ($p < 0.05$) from controls.

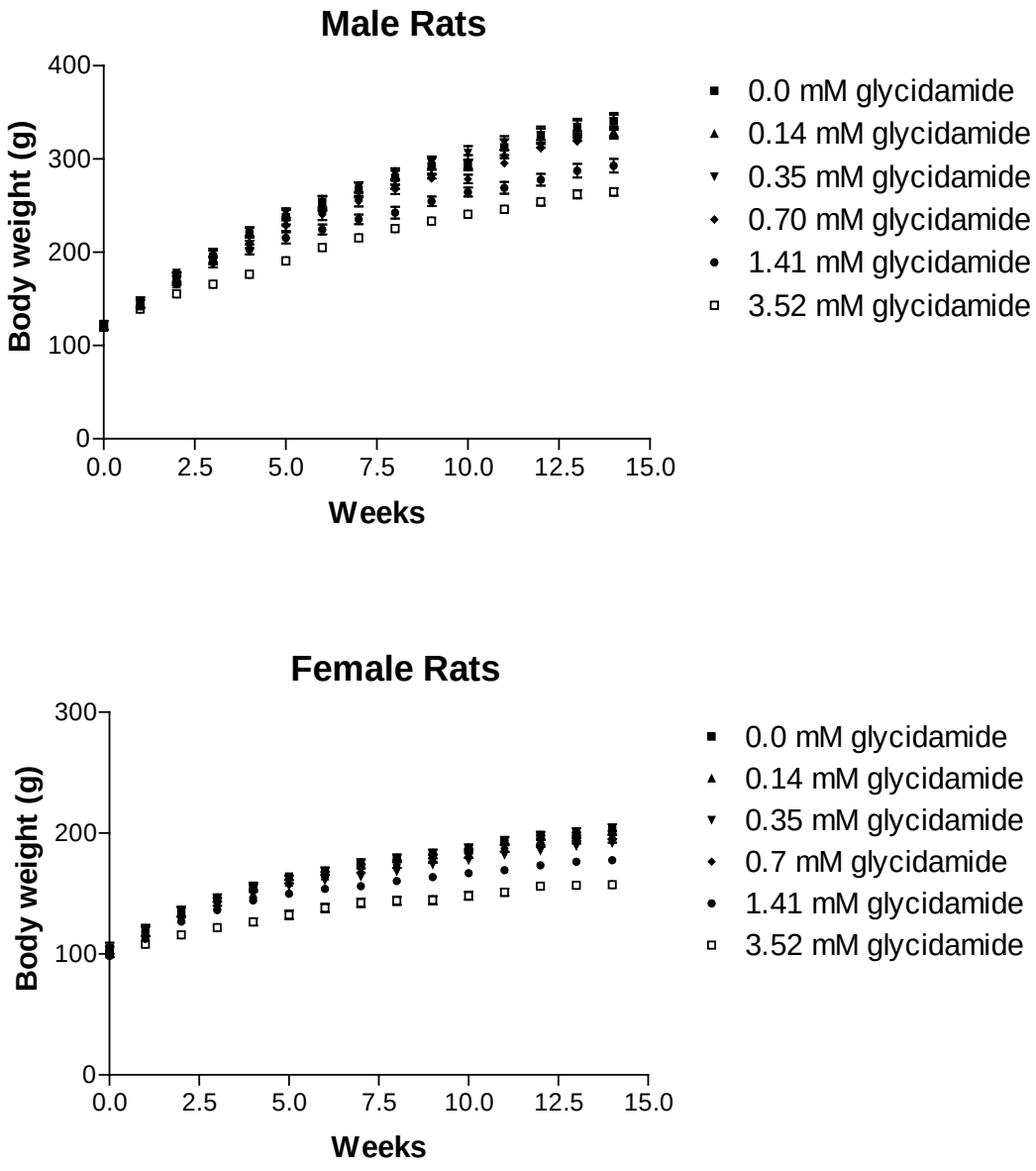


Figure 5. Growth Curves for Male and Female Rats in the Three-month Drinking Water Study of Glycidamide

Necropsy body weights and liver weights were decreased in male rats administered 1.41 and 3.52 mM glycidamide in the drinking water for 3 months (Table E-2). Brain weights were decreased in male rats given 3.52 mM glycidamide and the liver-weight-to-brain-weight ratios were decreased in male rats given 1.41 mM glycidamide (Table E-2). The liver weights were decreased at all dose levels in female rats administered glycidamide in the drinking water, brain weights were decreased at 0.70, 1.41, and 3.52 mM glycidamide, and the liver-weight-to-brain-weight ratios were decreased at 0.35 and 1.41 mM glycidamide (Table E-2).

There were significant dose effects in water consumption in male and female rats given glycidamide, and pairwise comparisons indicated significant decreases in water consumption over the 3-month treatment period at 1.41 and 3.52 mM glycidamide (Table 7).

There were significant dose effects in food consumption in male and female rats administered glycidamide in the drinking water. Pairwise comparisons indicated a significant decrease in food consumption over the 3-month treatment period in male but not female rats given 3.52 mM glycidamide (Table 8).

Male rats administered 0.14, 0.35, 0.70, 1.41, or 3.52 mM glycidamide in the drinking water consumed approximately 1.0, 2.4, 5.0, 10.1, or 26.9 mg glycidamide per kg body weight per day; the comparable values for female rats were 1.3, 3.4, 6.6, 13.5, or 33.8 mg glycidamide per kg body weight per day.

The only gross observation that was considered to be treatment-related was marked dilatation of the urinary bladder of two of eight male rats administered 3.52 mM glycidamide (Table 5). These animals had a clinical observation of paresis of the hind legs.

In rats administered glycidamide in the drinking water, treatment-related changes were observed in the following target tissues: sciatic nerve, spinal cord, spleen, testes, and epididymis. These tissues were examined microscopically in progressively lower dose groups until a no-observed-effect level was reached. The most significant treatment-related changes were peripheral neuropathy involving the sciatic nerve in one of eight female rats administered 3.52 mM glycidamide, and myelopathy of the lumbar spinal cord of three of eight female rats and two of eight male rats administered 3.52 mM glycidamide (Table 5). These degenerative changes were characterized by nerve fiber degeneration with dilatation and vacuolization of myelin sheaths along with swollen and shrunken axons. The severity of these changes was minimal to moderate. Luminal dilation of the urinary bladder was also diagnosed in the same male rats.

Testicular germ cell degeneration occurred in all male rats in the 0.70, 1.41, and 3.52 mM glycidamide dose groups, in three of eight male rats given 0.35 mM glycidamide, and in two of eight male rats given 0.14 mM glycidamide (Table 5). The severity of the degenerative change was moderate to marked in the 1.41 and 3.52 mM glycidamide groups and mild to minimal in 0.14, 0.35, and 0.70 mM glycidamide groups (Table 5). A corresponding lesion that consisted of exfoliated degenerating germ cells, cellular debris, and hypospermia was observed in the epididymides of these rats (Table 5).

Exposure Concentration Selection Rationale for Two-year Drinking Water Study: The selection of doses for the 2-year glycidamide drinking water study was based upon the effects observed in the 3-month drinking water studies with glycidamide (this study) and acrylamide[4], which were conducted simultaneously. Both 3.52 mM glycidamide and 3.52 mM acrylamide in drinking water resulted in hind limb paresis and decreased body weight. Decreases in body weight were also observed with 1.41 mM glycidamide and 1.41 mM acrylamide. In addition, hind limb paresis also occurred in female rats administered 1.41 mM acrylamide. Since one of the goals of this study was to compare acrylamide with glycidamide, a high dose of 0.70 mM glycidamide (61.2 ppm glycidamide) was selected for the chronic 2-year drinking water study, with the remaining doses being 0.0, 0.0875, 0.175, or 0.35 mM glycidamide (0, 7.65, 15.3, or 30.6 ppm glycidamide). These doses were identical to those used in the 2-year study with acrylamide[4].

Table 7. Water Consumption of Rats in the Three-month Drinking Water Study of Glycidamide[a]

Week	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM
Male						
2	20.9 ± 0.8	20.7 ± 0.8	21.2 ± 0.8	19.9 ± 0.8	21.1 ± 0.8	20.5 ± 0.8
3	20.5 ± 0.8	20.6 ± 0.8	21.7 ± 0.8	20.9 ± 0.8	21.9 ± 0.8	21.3 ± 0.8
4	22.8 ± 0.8	18.3 ± 0.8	19.5 ± 0.8	18.4 ± 0.8	19.4 ± 0.8	17.9 ± 0.8
5	22.3 ± 0.8	20.5 ± 0.8	21.3 ± 0.8	21.5 ± 0.8	20.4 ± 0.8	19.8 ± 0.8
6	20.1 ± 0.8	19.6 ± 0.8	19.3 ± 0.8	18.6 ± 0.8	17.3 ± 0.8	16.9 ± 0.8
7	20.4 ± 0.8	19.8 ± 0.8	21.6 ± 0.8	20.4 ± 0.8	17.0 ± 0.8	17.3 ± 0.8
8	20.3 ± 0.8	20.7 ± 0.8	20.6 ± 0.8	20.2 ± 0.8	16.4 ± 0.8	17.0 ± 0.8
9	20.7 ± 0.8	19.9 ± 0.8	20.1 ± 0.8	18.8 ± 0.8	17.4 ± 0.8	18.1 ± 0.8
10	21.9 ± 0.8	19.7 ± 0.8	20.9 ± 0.8	19.7 ± 0.8	18.1 ± 0.8	16.1 ± 0.8
11	21.3 ± 0.8	20.9 ± 0.8	21.2 ± 0.8	19.8 ± 0.8	18.4 ± 0.8	16.9 ± 0.8
12	23.2 ± 0.8	20.2 ± 0.8	20.1 ± 0.8	22.1 ± 0.8	18.9 ± 0.8	17.5 ± 0.8
13	21.4 ± 0.8	19.7 ± 0.8	21.4 ± 0.8	19.5 ± 0.8	19.1 ± 0.8	18.1 ± 0.8
Female						
2	20.5 ± 0.7	18.7 ± 0.7	18.3 ± 0.7	19.9 ± 0.7	18.4 ± 0.7	19.2 ± 0.7
3	19.4 ± 0.7	19.4 ± 0.7	19.1 ± 0.7	20.0 ± 0.7	19.7 ± 0.7	19.4 ± 0.7
4	18.4 ± 0.7	19.0 ± 0.7	18.4 ± 0.7	18.8 ± 0.7	18.8 ± 0.7	16.6 ± 0.7
5	19.5 ± 0.7	17.9 ± 0.7	20.1 ± 0.7	17.6 ± 0.7	18.4 ± 0.7	16.8 ± 0.7
6	17.1 ± 0.7	17.0 ± 0.7	17.6 ± 0.7	17.9 ± 0.7	16.2 ± 0.7	14.7 ± 0.7
7	19.3 ± 0.7	17.6 ± 0.7	17.2 ± 0.7	17.7 ± 0.7	16.2 ± 0.7	13.9 ± 0.7
8	18.3 ± 0.7	18.0 ± 0.7	18.5 ± 0.7	19.3 ± 0.7	16.5 ± 0.7	13.8 ± 0.7
9	17.6 ± 0.7	16.4 ± 0.7	16.9 ± 0.7	16.8 ± 0.7	15.5 ± 0.7	13.0 ± 0.7
10	17.3 ± 0.7	16.7 ± 0.7	17.2 ± 0.7	15.8 ± 0.7	15.6 ± 0.7	13.1 ± 0.7
11	17.5 ± 0.7	17.0 ± 0.7	16.3 ± 0.7	16.3 ± 0.7	15.6 ± 0.7	13.7 ± 0.7
12	17.7 ± 0.7	17.2 ± 0.7	17.9 ± 0.7	16.5 ± 0.7	15.9 ± 0.7	13.3 ± 0.7
13	17.9 ± 0.7	17.2 ± 0.7	16.1 ± 0.7	18.7 ± 0.7	16.3 ± 0.7	14.1 ± 0.7

[a]Water consumption is given as LS mean ± population standard error of the mean and is expressed as grams per animal per day.

Table 8. Food Consumption of Rats in the Three-month Drinking Water Study of Glycidamide[a]

Week	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM
Male						
3	15.1 ± 0.5	14.4 ± 0.5	16.4 ± 0.5	15.5 ± 0.5	16.2 ± 0.5	14.9 ± 0.5
4	15.7 ± 0.5	13.7 ± 0.5	15.1 ± 0.5	14.8 ± 0.5	15.7 ± 0.5	13.4 ± 0.5
5	16.0 ± 0.5	16.8 ± 0.5	16.4 ± 0.5	15.5 ± 0.5	16.3 ± 0.5	14.6 ± 0.5
6	16.2 ± 0.5	15.2 ± 0.5	15.7 ± 0.5	14.9 ± 0.5	14.1 ± 0.5	14.8 ± 0.5
7	16.3 ± 0.5	15.7 ± 0.5	16.7 ± 0.5	15.3 ± 0.5	14.4 ± 0.5	14.3 ± 0.5
8	15.3 ± 0.5	15.9 ± 0.5	16.5 ± 0.5	15.7 ± 0.5	13.9 ± 0.5	14.3 ± 0.5
9	16.9 ± 0.5	16.2 ± 0.5	16.5 ± 0.5	15.4 ± 0.5	14.2 ± 0.5	14.2 ± 0.5
10	17.3 ± 0.5	17.7 ± 0.5	16.8 ± 0.5	16.2 ± 0.5	15.8 ± 0.5	15.1 ± 0.5
11	15.8 ± 0.5	17.2 ± 0.5	17.7 ± 0.5	14.5 ± 0.5	16.0 ± 0.5	14.9 ± 0.5
12	20.2 ± 0.5	17.6 ± 0.5	17.5 ± 0.5	18.5 ± 0.5	15.0 ± 0.5	15.1 ± 0.5
13	17.7 ± 0.5	17.7 ± 0.5	18.8 ± 0.5	18.1 ± 0.5	17.6 ± 0.5	19.2 ± 0.5
Female						
3	12.2 ± 0.4	12.0 ± 0.4	11.9 ± 0.4	13.5 ± 0.4	11.9 ± 0.4	11.0 ± 0.4
4	11.5 ± 0.4	11.2 ± 0.4	11.9 ± 0.4	11.8 ± 0.4	13.0 ± 0.4	10.3 ± 0.4
5	12.3 ± 0.4	12.8 ± 0.4	12.9 ± 0.4	13.4 ± 0.4	12.0 ± 0.4	11.9 ± 0.4
6	11.8 ± 0.4	12.4 ± 0.4	13.2 ± 0.4	11.8 ± 0.4	11.8 ± 0.4	12.4 ± 0.4
7	11.3 ± 0.4	12.5 ± 0.4	12.8 ± 0.4	11.6 ± 0.4	11.5 ± 0.4	11.7 ± 0.4
8	11.8 ± 0.4	12.4 ± 0.4	12.1 ± 0.4	12.5 ± 0.4	10.8 ± 0.4	11.0 ± 0.4
9	11.6 ± 0.4	12.8 ± 0.4	12.3 ± 0.5	12.1 ± 0.5	11.9 ± 0.4	11.0 ± 0.4
10	12.2 ± 0.4	13.6 ± 0.4	13.2 ± 0.4	12.8 ± 0.4	11.6 ± 0.4	11.8 ± 0.4
11	11.2 ± 0.4	11.9 ± 0.4	12.4 ± 0.4	11.6 ± 0.4	12.3 ± 0.4	11.0 ± 0.4
12	13.4 ± 0.4	12.9 ± 0.4	13.0 ± 0.4	13.2 ± 0.4	12.2 ± 0.4	11.2 ± 0.4
13	13.3 ± 0.4	12.5 ± 0.4	13.6 ± 0.4	14.1 ± 0.4	13.7 ± 0.4	12.2 ± 0.4

[a]Food consumption is given as LS mean ± population standard error of the mean and is expressed as grams per animal per day.

Two-year Study

Survival and Cause of Death

There was a dose-related trend in survival in male and female F344/N Nctr rats given glycidamide in the drinking water (Table 9 and Figure 6). Compared to control rats, both male and female rats administered 0.35 and 0.70 mM glycidamide had decreased survival. The primary cause for the early removal or death of male rats was neoplasms, including mononuclear cell leukemia, pituitary gland adenoma or carcinoma, preputial gland carcinoma, thyroid gland adenoma or carcinoma, and Zymbal's gland carcinoma. The primary cause for the early removal or death of female rats was neoplasms, including mononuclear cell leukemia, mammary gland fibroadenoma, clitoral gland adenoma or carcinoma, pituitary gland adenoma, and Zymbal's gland carcinoma.

Table 9. Survival and Disposition of Rats in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Male					
Animals initially in study	48	48	48	48	48
Wrong sex[a]	0	0	0	1	0
Moribund	23	18	28	36	43
Natural deaths	4	12	5	4	3
Animals surviving to study termination[a]	21	18	15	7	2
Percent probability of survival at end of study[b]	44	38	31	15	4
Mean survival (weeks)[c]	94.7	92.6	93.7	92.1	84.1
Survival analysis[d]	P < 0.001	P = 0.418	P = 0.199	P = 0.006	P < 0.001
Female					
Animals initially in study	48	48	48	48	48
Moribund	10	21	16	28	43
Natural deaths	3	1	5	3	3
Animals surviving to study termination[a]	35	26	27	17	2
Percent probability of survival at end of study[b]	73	54	58	35	4
Mean survival (weeks)[c]	98.8	97.2	97.5	91.6	81.1
Survival analysis[d]	P < 0.001	P = 0.070	P = 0.101	P < 0.001	P < 0.001

[a]Censored from the survival analyses.
[b]Kaplan-Meier survival estimates.
[c]Mean of all deaths (censored and uncensored).
[d]The result of the trend test is in the 0.0 mM glycidamide column, and the results of the pairwise comparisons[62] with the 0.0 mM glycidamide are in the treatment group columns. P-values <0.05 were considered significant.

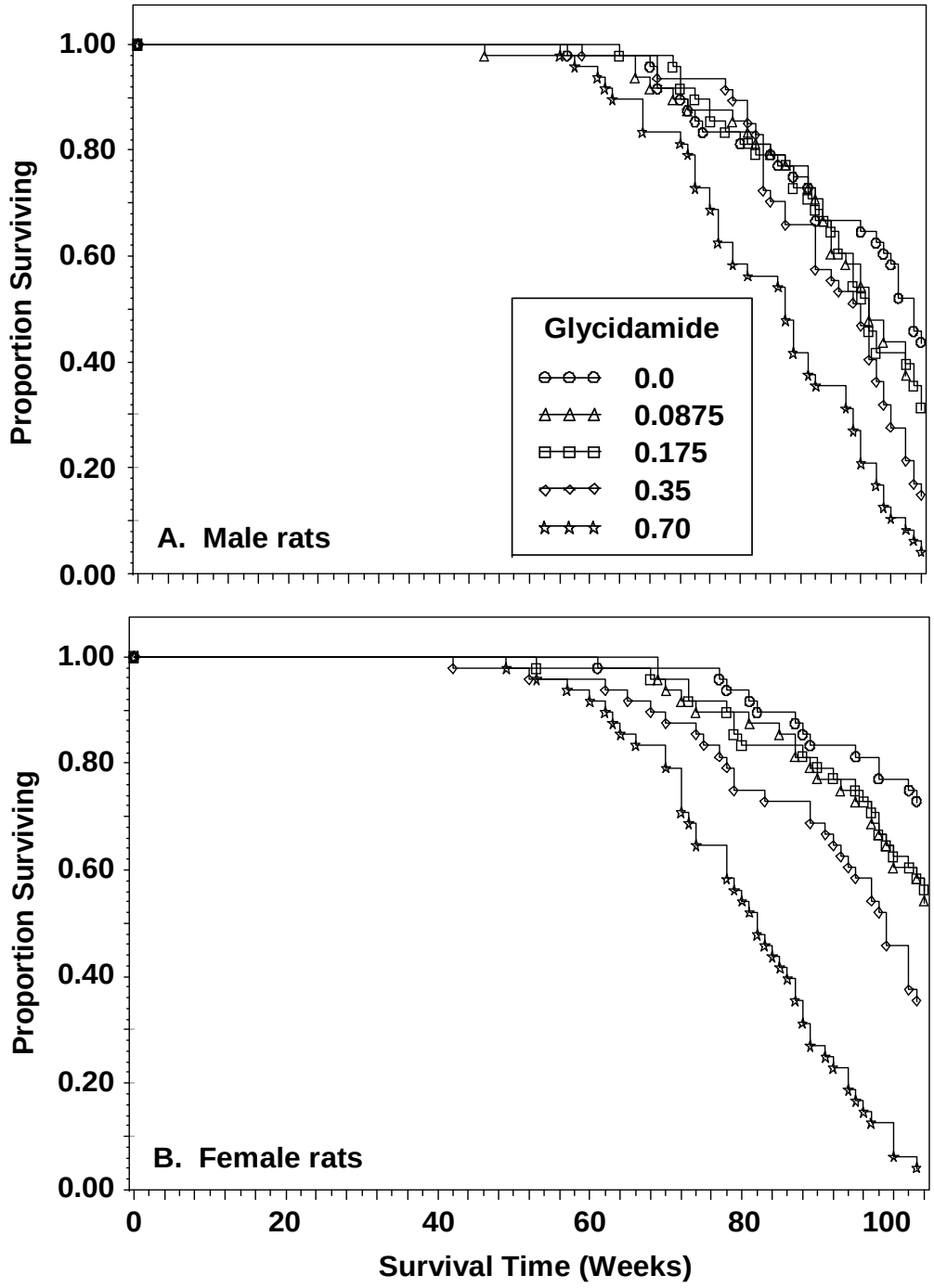


Figure 6. Kaplan-Meier Survival Curves for Male and Female Rats Administered Glycidamide (mM) in Drinking Water for Two Years

Body Weights and Food and Water Consumption

There were significant dose-related trends in body weights beginning at 8 weeks in male F344/N Nctr rats administered glycidamide in the drinking water (Figure 7 and Table 10). Pairwise comparisons indicated that treatment with 0.70 mM glycidamide resulted in significant decreases

in body weight gain beginning at week 8 in the male rats; significant decreases in body weight gain occurred sporadically in male rats given 0.35 mM glycidamide (Figure 7 and Table 10).

In female rats, there were significant dose-related trends beginning at 4 weeks (Figure 7 and Table 11). In female rats, pairwise comparisons indicated that treatment with 0.175, 0.35, or 0.70 mM glycidamide resulted in significant decreases in body weight gain beginning at 4 weeks (Figure 7 and Table 11); significant decreases in body weight gain were also observed at 0.0875 mM glycidamide at 84 weeks and at 92 to 104 weeks. At the end of the 104-week period, the male rats administered 0.70 mM glycidamide weighed 82% of the control group; the female rats administered 0.70 mM glycidamide weighed 79% of the control group.

Glycidamide in the drinking water resulted in sporadic dose-related trends in food consumption in male (Table G-1) and female (Table G-2) F344/N Nctr rats.

Glycidamide in the drinking water resulted in sporadic dose-related trends in water consumption in male (Table 12) and female (Table 13) F344/N Nctr rats.

The mean glycidamide exposure for rats, calculated at 4-week intervals, is presented in Figure 8 and Table 12 and Table 13. The mean amount of glycidamide consumed by male rats for the entire 2-year experiment was 0.39, 0.79, 1.56, and 3.34 mg glycidamide per kg body weight per day for the 0.0875, 0.175, 0.35, and 0.70 mM glycidamide dose groups, respectively (Figure 8 and Table 12). The corresponding values for female rats were 0.54, 1.08, 2.23, and 4.65 mg glycidamide per kg body weight per day (Figure 8 and Table 13).

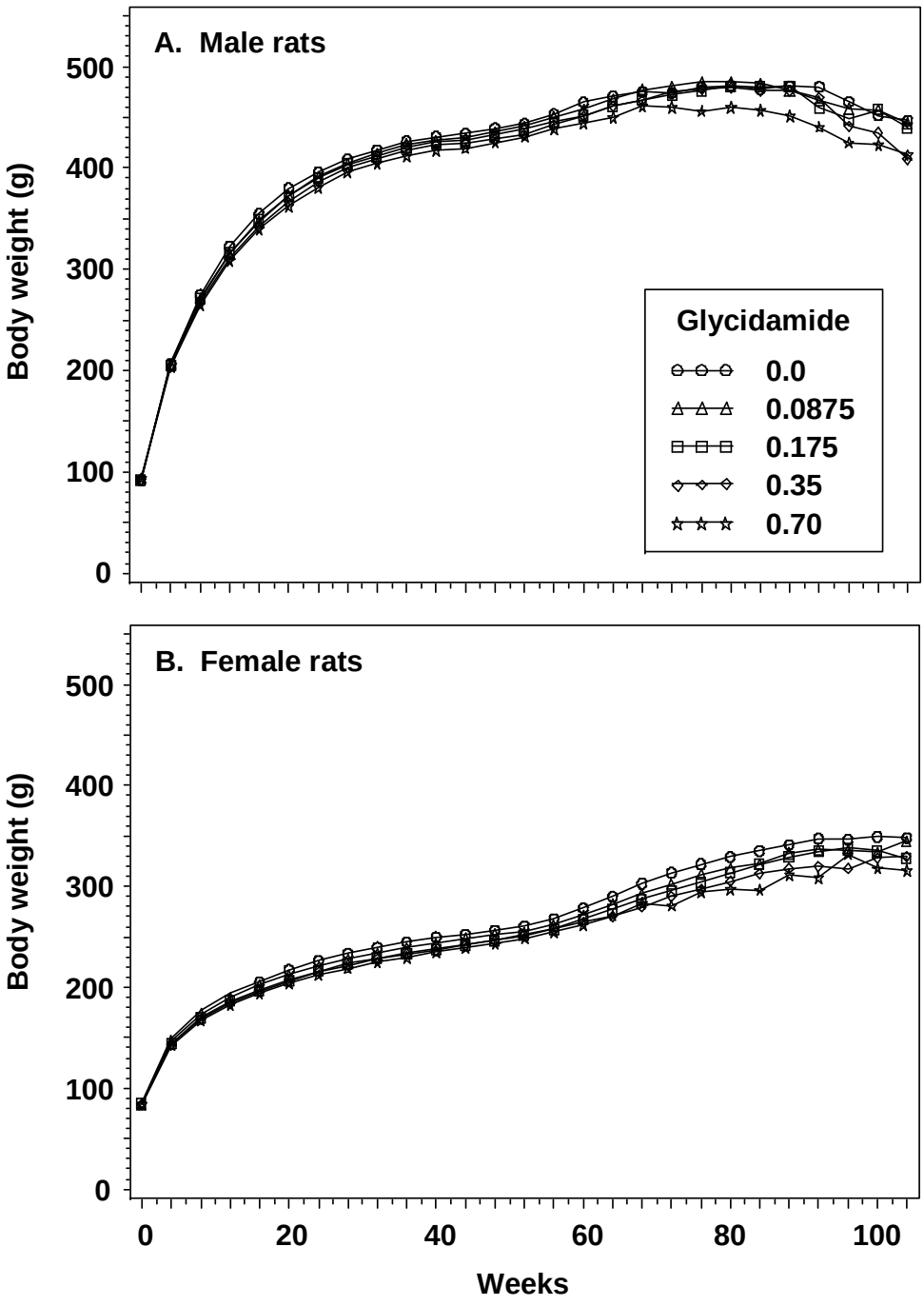


Figure 7. Growth Curves for Male and Female Rats Administered Glycidamide (mM) in Drinking Water for Two Years

Table 10. Mean Body Weights[a] and Survival of Male Rats in the Two-year Drinking Water Study of Glycidamide

Weeks on Study	0 mM		0.0875 mM			0.175 mM			0.35 mM			0.70 mM		
	Mean Wt. (g)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors
4	206.8	48	206.4	99.8	48	205.5	99.4	48	203.4	98.4	47	203.8	98.5	48
8	275.2*	48	272.0	98.8	48	271.2	98.5	48	268.4	97.5	47	265.1*	96.3	48
12	323.0*	48	316.1	97.9	48	316.4	98.0	48	311.8	96.5	47	308.3*	95.5	48
16	354.8*	48	347.6	98.0	48	348.6	98.3	48	341.9	96.4	47	340.0*	95.8	48
20	380.0*	48	372.8	98.1	48	372.2	98.0	48	366.6	96.5	47	362.1*	95.3	48
24	395.9*	48	392.0	99.0	48	390.4	98.6	48	386.5	97.6	47	381.0*	96.2	48
28	408.8*	48	405.0	99.1	48	404.0	98.8	48	400.5	98.0	47	395.8*	96.8	48
32	417.5*	48	414.9	99.4	48	412.1	98.7	48	408.5	97.8	47	404.7*	96.9	48
36	426.6*	48	423.7	99.3	48	420.3	98.5	48	418.1	98.0	47	411.7*	96.5	48
40	430.9*	48	427.1	99.1	48	425.9	98.8	48	422.8	98.1	47	417.2*	96.8	48
44	434.0*	48	429.8	99.0	48	426.9	98.4	48	424.5	97.8	47	419.3*	96.6	48
48	439.3*	48	434.2	98.8	47	433.2	98.6	48	428.6*	97.6	47	425.2*	96.8	48
52	443.8*	48	439.4	99.0	47	438.8	98.9	48	432.8	97.5	47	430.8*	97.1	48
56	453.2*	48	449.0	99.1	47	445.2	98.2	48	442.2	97.6	47	439.1*	96.9	47
60	463.5*	47	457.6	98.7	47	451.9	97.5	48	450.5*	97.2	46	444.5*	95.9	46
64	469.6*	47	466.6	99.4	47	461.3	98.2	47	460.0	97.9	46	448.3*	95.5	43
68	474.5*	47	476.8	100.5	44	467.6	98.6	47	466.4	98.3	46	457.5*	96.4	40
72	472.4*	44	481.1	101.8	43	471.9	99.9	44	474.2	100.4	44	456.2*	96.6	39
76	475.8*	40	484.9	101.9	42	475.1	99.8	43	476.7	100.2	44	451.5*	94.9	35
80	478.3*	40	484.7	101.3	41	476.5	99.6	40	477.6	99.9	42	449.7*	94.0	28
84	474.0*	39	482.3	101.7	39	474.1	100.0	38	469.3	99.0	34	445.7*	94.0	27
88	472.5*	36	472.2	99.9	37	471.4	99.7	35	463.5	98.1	31	436.9*	92.5	20
92	470.1*	32	458.1	97.5	32	449.9	95.7	32	450.2	95.8	26	423.8*	90.2	17
96	455.9*	32	436.5	95.7	28	428.5	94.0	26	418.7*	91.8	23	394.0*	86.4	11
100	440.0*	29	428.2	97.3	21	426.3	96.9	20	407.2*	92.5	14	381.4*	86.7	6
104	428.0*	22	411.4	96.1	18	410.9	96.0	17	375.4*	87.7	8	352.2*	82.3	2
Mean for Weeks														
4–104	421.7		418.1			414.5			409.5			397.9		

[a]An * in the 0.0 mM glycidamide column indicates a significant dose-related trend ($p < 0.05$); an * in the treatment column indicates a significant ($p < 0.05$) pairwise comparison of the dose group to the 0.0 mM glycidamide group as determined by Dunnett's test.

Table 11. Mean Body Weights[a] and Survival of Female Rats in the Two-year Drinking Water Study of Glycidamide

Weeks on Study	0 mM		0.0875 mM			0.175 mM			0.35 mM			0.70 mM		
	Mean Wt. (g)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors
4	149.8*	48	146.9	98.1	48	143.9*	96.1	48	142.9*	95.4	48	142.5*	95.1	48
8	176.8*	48	173.7	98.3	48	170.1*	96.2	48	168.3*	95.2	48	167.7*	94.9	48
12	194.7*	48	190.2	97.7	48	186.6*	95.8	48	183.8*	94.4	48	183.1*	94.1	48
16	205.4*	48	202.4	98.5	48	197.5*	96.1	48	196.1*	95.5	48	193.7*	94.3	48
20	217.5*	48	212.9	97.9	48	207.1*	95.2	48	206.1*	94.8	48	203.9*	93.8	48
24	226.6*	48	220.8	97.4	48	216.1*	95.4	48	214.9*	94.8	48	212.3*	93.7	48
28	233.8*	48	228.0	97.5	48	223.4*	95.5	48	221.8*	94.8	48	218.8*	93.6	48
32	239.9*	48	234.2	97.6	48	228.7*	95.4	48	228.1*	95.1	48	224.9*	93.8	48
36	244.8*	48	239.0	97.6	48	233.6*	95.4	48	232.8*	95.1	48	229.5*	93.7	48
40	249.8*	48	243.4	97.5	48	237.6*	95.1	48	236.8*	94.8	48	234.9*	94.0	48
44	252.3*	48	248.3	98.4	48	241.8*	95.8	48	241.1*	95.5	47	239.6*	95.0	48
48	256.7*	48	252.9	98.5	48	246.7*	96.1	48	245.4*	95.6	47	243.1*	94.7	48
52	260.7*	48	255.2	97.9	48	251.3*	96.4	48	250.8*	96.2	46	247.6*	95.0	47
56	268.0*	48	262.4	97.9	48	257.8*	96.2	47	256.9*	95.9	46	254.3*	94.9	46
60	278.4*	48	271.4	97.5	48	267.1*	95.9	47	263.8*	94.8	46	261.7*	94.0	45
64	289.6*	47	282.4	97.5	48	276.7*	95.5	47	269.9*	93.2	45	269.5*	93.1	42
68	302.9*	47	293.0	96.7	48	287.0*	94.7	47	278.8*	92.1	44	281.5*	92.9	40
72	313.4*	47	300.9	96.0	45	295.0*	94.1	46	287.3*	91.7	42	279.7*	89.2	38
76	321.9*	47	309.2	96.1	43	302.6*	94.0	44	294.9*	91.6	40	285.9*	88.8	31
80	328.7*	45	315.5	96.0	43	310.0*	94.3	41	299.8*	91.2	36	288.6*	87.8	27
84	334.7*	43	319.3*	95.4	42	315.9*	94.4	40	306.9*	91.7	35	284.8*	85.1	22
88	340.0*	42	325.4	95.7	39	323.3*	95.1	40	312.6*	91.9	35	288.3*	84.8	17
92	344.6*	40	326.5*	94.8	37	325.3*	94.4	38	315.5*	91.6	32	283.4*	82.2	12
96	345.0*	39	324.9*	94.2	35	326.0*	94.5	35	312.8*	90.7	28	292.2*	84.7	8
100	347.1*	37	318.9*	91.9	31	322.6*	92.9	31	313.6*	90.3	22	274.8*	79.2	6
104	347.2*	35	326.5*	94.0	27	319.8*	92.1	28	314.1*	90.5	17	275.5*	79.4	2
Mean for Weeks														
4–104	271.9		262.5			258.2			253.7			244.7		

[a]An * in the 0.0 mM glycidamide column indicates a significant dose-related trend ($p < 0.05$); an * in the treatment column indicates a significant ($p < 0.05$) pairwise comparison of the dose group to the 0.0 mM glycidamide group as determined by Dunnett's test.

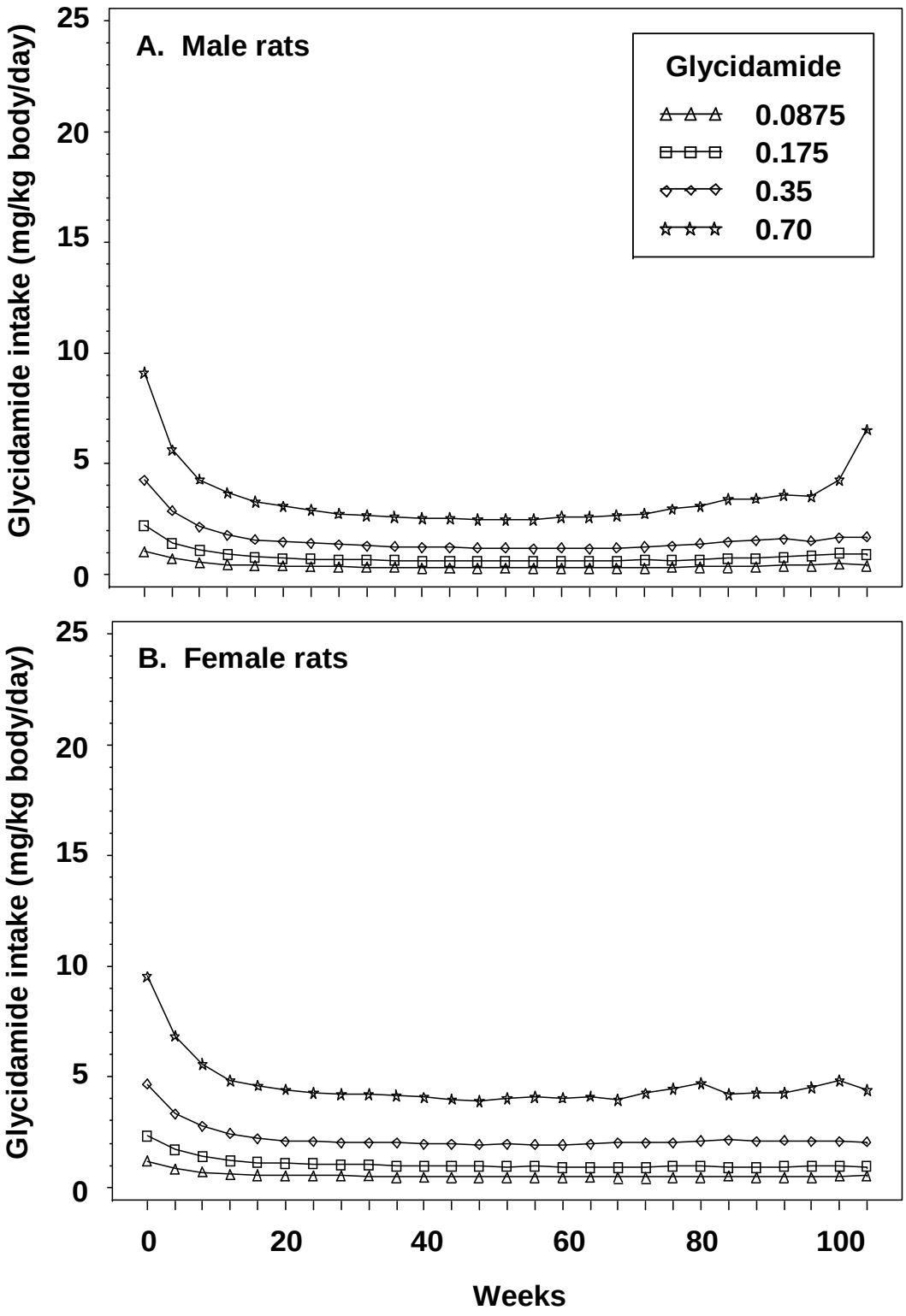


Figure 8. Glycidamide Intake in Male and Female Rats Administered Glycidamide (mM) in Drinking Water for Two Years

Table 12. Water and Glycidamide Consumption by Male Rats in the Two-year Drinking Water Study of Glycidamide

Week[a]	0 mM	0.0875 mM		0.175 mM		0.35 mM		0.70 mM	
	Water (g/day)	Water (g/day)	Dose[b]	Water (g/day)	Dose	Water (g/day)	Dose	Water (g/day)	Dose
4	21.3	21.3	0.70	21.5	1.42	21.4	2.86	20.9	5.62
8	20.2	20.0	0.52	20.3	1.07	20.2	2.16	19.8	4.29
12	19.4	18.9	0.44	19.3	0.89	18.8	1.77	19.3	3.68
16	18.8	18.2	0.39	18.6	0.79	18.0	1.57	18.8	3.28
20	18.5	18.1	0.36	18.5	0.74	18.1	1.47	18.7	3.07
24	18.0	17.7	0.34	18.1	0.69	18.0	1.40	18.3	2.89
28	17.6	17.8	0.33	17.7	0.66	17.6	1.33	17.9	2.74
32	17.5	17.5	0.32	17.7	0.65	17.5	1.29	17.8	2.66
36	17.2	17.4	0.31	17.4	0.63	17.3	1.25	17.7	2.60
40	17.1	17.0	0.30	17.2	0.62	17.1	1.22	17.4	2.53
44	17.1	16.7	0.30	17.2	0.61	16.8	1.20	17.5	2.54
48	16.9	16.7	0.29	16.9	0.59	16.8	1.18	17.3	2.48
52	17.4	16.9	0.29	17.4	0.60	17.0	1.19	17.6	2.48
56	17.7	17.2	0.29	17.6	0.60	17.2	1.17	17.6	2.47
60	17.7*	17.3	0.29	17.7	0.59	17.7	1.19	18.7	2.59
64	18.3	17.7	0.29	18.3	0.61	17.9	1.17	18.9	2.59
68	18.6	18.2	0.29	18.4	0.60	18.2	1.19	19.5	2.64
72	19.3	19.1	0.30	19.3	0.64	19.5	1.24	19.8	2.73
76	20.8	20.6	0.32	19.4	0.63	20.2	1.29	21.5	2.94
80	21.6	21.5	0.34	20.9	0.68	21.4	1.38	22.6	3.07
84	22.5*	22.0	0.35	22.1	0.72	22.4	1.46	24.3	3.40
88	23.7	22.3	0.36	22.4	0.74	23.7	1.54	24.7	3.42
92	23.9	23.1	0.39	22.6	0.77	23.8	1.58	24.6	3.57
96	24.8	24.5	0.42	24.0	0.84	19.2*	1.49	22.6	3.51
100	27.3*	26.2	0.47	25.9	0.94	20.0*	1.64	21.0	4.24
104	30.9	26.2	0.41	28.6	0.92	19.7*	1.68	25.9	6.54
Mean for Weeks									
4–104	20.1	19.6	0.39	19.8	0.79	19.1	1.56	20.0	3.34

[a]Week indicates the last week of a 4-week interval of daily water consumption, measured weekly by cage.
[b]Dose is expressed as the mean value measured in mg/kg body weight/day.
*An * in the 0.0 mM glycidamide column indicates a significant dose-related trend ($p < 0.05$); an * in the treatment column indicates a significant ($p < 0.05$) pairwise comparison of the dose group to the 0.0 mM glycidamide group as determined by Dunnett's test.

Table 13. Water and Glycidamide Consumption by Female Rats in the Two-year Drinking Water Study of Glycidamide

Week[a]	0 mM	0.0875 mM		0.175 mM		0.35 mM		0.70 mM	
	Water (g/day)	Water (g/day)	Dose[b]	Water (g/day)	Dose	Water (g/day)	Dose	Water (g/day)	Dose
4	17.6	17.5	0.84	17.3	1.71	16.8	3.34	17.1	6.84
8	16.4	16.1	0.68	16.2	1.41	15.7	2.76	15.8	5.56
12	15.7*	15.1	0.59	15.3	1.23	15.0	2.43	14.8	4.84
16	15.3	15.1	0.56	14.9	1.13	14.7	2.23	14.9	4.60
20	15.0	14.9	0.53	14.9	1.08	14.5	2.10	15.0	4.42
24	15.0	15.1	0.52	15.2	1.06	14.9	2.09	15.0	4.28
28	15.3	15.4	0.51	15.2	1.03	15.0	2.04	15.3	4.22
32	15.6	15.6	0.50	15.4	1.02	15.4	2.05	15.6	4.20
36	15.7	15.5	0.49	15.3	0.99	15.5	2.02	15.7	4.14
40	15.7	15.5	0.48	15.2	0.97	15.4	1.97	15.7	4.06
44	15.6	15.9	0.49	15.4	0.97	15.7	1.97	15.7	3.99
48	15.6	15.9	0.48	15.5	0.95	15.7	1.93	15.7	3.91
52	15.8	16.1	0.48	15.5	0.94	16.3	1.98	16.4	4.03
56	16.5*	16.4	0.47	16.2	0.95	16.5	1.94	17.2	4.09
60	16.6	16.8	0.47	16.4	0.93	16.8	1.92	17.3	4.04
64	17.1*	17.1	0.45	16.9	0.92	17.5	1.96	18.2	4.11
68	17.6	17.8	0.47	17.1	0.91	18.5	2.01	18.4	3.96
72	18.1*	18.4	0.47	17.7	0.92	19.2	2.01	19.2	4.24
76	18.4*	19.1	0.47	18.5	0.96	19.8	2.04	20.6*	4.44
80	19.2*	20.0	0.49	19.6	0.95	21.0	2.11	22.6*	4.69
84	19.5*	20.7	0.50	19.9	0.93	21.9*	2.16	21.8	4.22
88	20.0	20.5	0.47	19.9	0.92	21.3	2.08	21.7	4.28
92	20.7	20.6	0.47	20.5	0.93	21.6	2.12	22.3	4.27
96	20.7*	21.0	0.48	20.7	0.96	21.1	2.09	24.1*	4.52
100	21.9	22.3	0.51	20.8	0.99	22.1	2.09	24.4	4.82
104	22.5	22.8	0.51	20.2	0.95	23.3	2.05	22.4	4.38
Mean for Weeks									
4–104	17.4	17.6	0.54	17.2	1.08	17.7	2.23	18.2	4.65

[a]Week indicates the last week of a 4-week interval of daily water consumption, measured weekly by cage.
[b]Dose is expressed as the mean value measured in mg/kg body weight/day.
In the 0.0 mM glycidamide column "" indicates a significant trend ($p < 0.05$); in the treatment column "*" indicates a significant ($p < 0.05$) pairwise comparison of the dose group to the 0.0 mM glycidamide group as determined by Dunnett's test.

Neoplastic Findings

Glycidamide in the drinking water resulted in thyroid gland neoplasms in male and female F334/N rats. In both sexes of rats, there was a dose-related increase in follicular cell adenoma, follicular cell carcinoma, and combined follicular cell adenoma or carcinoma (Table 14). In male rats, the incidence of follicular cell adenoma, follicular cell carcinoma, and combined follicular cell adenoma or carcinoma was significantly increased at 0.70 mM glycidamide. In female rats, the incidence of follicular cell adenoma was significantly increased in the 0.70 mM glycidamide dose group and the incidence of combined follicular cell adenoma or carcinoma was significant at 0.175, 0.35, and 0.70 mM glycidamide.

Morphologically, both follicular cell adenomas and follicular cell carcinomas were typical of spontaneous thyroid follicular neoplasms in F344/N Nctr rats. Adenomas were small circumscribed solitary lesions that slightly compressed adjacent parenchyma. The well-differentiated follicular cells were usually arranged in a single layer on the basement membrane. Adenomas were arranged in either a follicular or a papillary pattern that sometimes included cystic structures. Follicular cell carcinomas were larger masses without definite boundaries and with disorganized growth patterns. The cells were pleomorphic, sometimes atypical, and were often piled in multiple layers or arranged in solid clusters or sheets, with invasion of the thyroid capsule or blood vessels.

The drinking water administration of glycidamide was associated with a dose-related increase in squamous cell papilloma of the oral mucosa and squamous cell papilloma of the tongue in male F344/N Nctr rats, squamous cell papilloma and squamous cell carcinoma of the oral mucosa in female F344/N Nctr rats, and combined squamous cell papilloma or carcinoma of the oral mucosa or tongue in both sexes (Table 15). The incidence of squamous cell papilloma of the tongue was significant in male rats at 0.70 mM glycidamide and the incidence of combined squamous cell papilloma or carcinoma of the oral mucosa or tongue was significant in both sexes at 0.70 mM glycidamide. Microscopically, the oral squamous cell papillomas were elevated nodules or masses consisting of several layers of well-differentiated squamous cells covering multiple projections of a fibrovascular core. The squamous cell carcinomas on the tongue were gross lesions situated on the dorsum of the tongue. Microscopic examination revealed that they consisted of atypical squamous cells that invaded tissues underlying the epithelium.

Both sexes of F344/N Nctr rats had dose related increases in the incidence of mononuclear cell leukemia, with the increase in incidence being significant at 0.70 mM glycidamide (Table 16).

The consumption of glycidamide in the drinking water was associated with development of malignant mesothelioma on membranes surrounding the epididymis and on testicular tunics in male rats (Table 17). The incidence of malignant mesothelioma was significantly increased in the epididymis, testes, and combined testes and epididymis in male rats administered 0.35 and 0.70 mM glycidamide. Microscopically, malignant mesotheliomas in the epididymis and testicular tunics were characterized by complex papillary surface growths of one to several layers of polyhedral to cuboidal mesothelial cells on pedunculated fibrovascular stalks. The neoplastic cells had either abundant weakly eosinophilic cytoplasm and ovoid nuclei with one or more nucleoli or scanty cytoplasm and numerous small basophilic nuclei.

Glycidamide in the drinking water resulted in dose-related increases in malignant schwannoma in the heart of male F344/N Nctr rats (Table 18), with the incidence being significant at 0.70 mM

glycidamide. The microscopic morphology of schwannomas in the heart was similar in lesions located in either subendocardial or intramural locations and consisted of spindeloid cells with fusiform granular to hyperchromatic nuclei and pale indistinct cytoplasm. The neoplastic cells were arranged in either discreet foci or more commonly infiltrated around and between adjacent myocardial fibers. The designation of malignancy was based primarily on the infiltrative characteristic.

Female F344/N Nctr rats exposed to glycidamide in the drinking water had an increased prevalence of fibroadenomas in the mammary gland, with the incidence in all dose groups being significantly increased compared to the control group (Table 19). Microscopically mammary gland fibroadenomas were characterized by variable amounts of uniform well-differentiated glandular tissue or epithelium embedded in dense mature fibrous connective tissue. The neoplasms were well circumscribed and well demarcated from adjacent tissue.

In female F344/N Nctr rats, there were dose-related increases in clitoral gland carcinoma, with the incidence being significantly increased at 0.35 and 0.70 mM glycidamide (Table 20). Acinar arrangement was typically not present in carcinomas; instead, the neoplastic cells were arranged in irregular lobules, cords, or sheets. The degree of cellular anaplasia varied. Female F344/N Nctr rats also had dose-related increases in squamous cell papilloma of the forestomach, with the incidence being significantly increased at 0.70 mM glycidamide (Table 21).

Table 14. Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Rats in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Male					
Follicular Cell Adenoma, Multiple[a]	0/47	0/42	0/48	0/47	1/46
Follicular Cell Adenoma (including multiple)[b]					
Overall rate[a]	2/47 (4%)	1/42 (2%)	3/48 (6%)	3/47 (6%)	8/46 (17%)
Poly-3 test[c]	P < 0.001	P = 0.534N	P = 0.494	P = 0.454	P = 0.011
Follicular Cell Carcinoma[d]					
Overall rate	0/47 (0%)	2/42 (5%)	3/48 (6%)	1/47 (2%)	5/46 (11%)
Poly-3 test	P = 0.014	P = 0.217	P = 0.114	P = 0.481	P = 0.011
Follicular Cell Adenoma or Carcinoma[e]					
Overall rate	2/47 (4%)	3/42 (7%)	6/48 (13%)	4/47 (9%)	13/46 (28%)
Poly-3 test	P < 0.001	P = 0.460	P = 0.130	P = 0.295	P < 0.001
Female					
Follicular Cell Adenoma[f]					
Overall rate	0/48 (0%)	3/48 (6%)	3/46 (7%)	1/46 (2%)	5/47 (11%)
Poly-3 test	P = 0.017	P = 0.109	P = 0.104	P = 0.459	P = 0.007
Follicular Cell Carcinoma, Multiple[a]	0/48	0/48	0/46	0/46	1/47
Follicular Cell Carcinoma (including multiple)[g]					
Overall rate	0/48 (0%)	0/48 (0%)	2/46 (4%)	3/46 (7%)	3/47 (6%)
Poly-3 test	P = 0.006	–	P = 0.219	P = 0.085	P = 0.051
Follicular Cell Adenoma or Carcinoma[h]					
Overall rate	0/48 (0%)	3/48 (6%)	5/46 (11%)	4/46 (9%)	8/47 (17%)
Poly-3 test	P < 0.001	P = 0.109	P = 0.025	P = 0.037	P < 0.001

[a]Number of animals with neoplasm per number of animals examined microscopically.
[b]The historical incidence of thyroid gland follicular cell adenoma in NCTR control male F344/N Nctr rats is 0.6% (range 0.0–4.2%; Table A-3) for all studies and 2.1% (range 0.0–4.2%; Table A-3) for drinking water studies.
[c]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.
[d]The historical incidence of thyroid gland follicular cell carcinoma in NCTR control male F344/N Nctr rats is 0.2% (range 0.0–2.1%; Table A-3) for all studies and 1.1% (range 0.0–2.1%; Table A-3) for drinking water studies.
[e]The historical incidence of thyroid gland follicular cell adenoma or carcinoma in NCTR control male F344/N Nctr rats is 0.8% (range 0.0–4.2%; Table A-3) for all studies and 3.2% (range 2.1–4.2%; Table A-3) for drinking water studies.
[f]The historical incidence of thyroid gland follicular cell adenoma in NCTR control female F344/N Nctr rats is 0.1% (range 0.0–2.9%; Table B-3) for all studies and 0.0% (Table B-3) for drinking water studies.
[g]The historical incidence of thyroid gland follicular cell carcinoma in NCTR control female F344/N Nctr rats is 0.0% (Table B-3) for all studies and 0.0% (Table B-3) for drinking water studies.
[h]The historical incidence of thyroid gland follicular cell adenoma or carcinoma in NCTR control female F344/N Nctr rats is 0.1% (range 0.0–2.9%; Table B-3) for all studies and 0.0% (Table B-3) for drinking water studies.

Table 15. Incidences of Neoplasms of the Oral Cavity in Rats in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Male					
Oral Mucosa, Squamous Cell Papilloma[c]					
Overall rate[a]	1/48 (2%)	1/48 (2%)	0/48 (0%)	2/47 (4%)	3/48 (6%)
Poly-3 test[b]	P = 0.050	P = 0.754	P = 0.509N	P = 0.462	P = 0.201
Oral Mucosa, Squamous Cell Carcinoma[d]					
Overall rate	1/48 (2%)	0/48 (0%)	1/48 (2%)	1/47 (2%)	0/48 (0%)
Poly-3 test	P = 0.489N	P = 0.509N	P = 0.751	P = 0.735	P = 0.568N
Tongue, Squamous Cell Papilloma[c]					
Overall rate	0/48 (0%)	1/48 (2%)	0/48 (0%)	1/47 (2%)	4/48 (8%)
Poly-3 test	P = 0.003	P = 0.493	–	P = 0.475	P = 0.031
Tongue, Squamous Cell Carcinoma[d]					
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)	0/47 (0%)	0/48 (0%)
Poly-3 test	P = 0.649N	–	P = 0.494	–	–
Oral Mucosa or Tongue, Squamous Cell Papilloma or Carcinoma[e]					
Overall rate	2/48 (4%)	2/48 (4%)	2/48 (4%)	3/47 (6%)	7/48 (15%)
Poly-3 test	P = 0.005	P = 0.686	P = 0.685	P = 0.454	P = 0.030
Female					
Oral Mucosa: Squamous Cell Papilloma[f]					
Overall rate	1/48 (2%)	1/48 (2%)	2/48 (4%)	0/48 (0%)	4/48 (8%)
Poly-3 test	P = 0.045	P = 0.747	P = 0.477	P = 0.539N	P = 0.070
Oral Mucosa: Squamous Cell Carcinoma[g]					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	2/48 (4%)
Poly-3 test	P = 0.015	–	–	P = 0.461	P = 0.145
Tongue: Squamous Cell Papilloma[f]					
Overall rate	0/48 (0%)	1/48 (2%)	0/48 (0%)	1/48 (2%)	0/48 (0%)
Poly-3 test	P = 0.548	P = 0.488	–	P = 0.464	–
Tongue: Squamous Cell Carcinoma[g]					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Poly-3 test	P = 0.105	–	–	–	P = 0.397
Oral Mucosa or Tongue: Squamous Cell Papilloma or Carcinoma[h]					
Overall rate	1/48 (2%)	2/48 (4%)	2/48 (4%)	2/48 (4%)	7/48 (15%)
Poly-3 test	P = 0.001	P = 0.481	P = 0.477	P = 0.437	P = 0.005

[a]Number of animals with neoplasm per number of animals examined microscopically.
[b]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test

accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.
[c]The historical incidence of squamous cell papilloma or papilloma in the oral cavity in NCTR control male F344/N Nctr rats is 0.3% (range 0.0–2.1%; Table A-7) for all studies and 1.0% (range 0.0–2.1%; Table A-7) for drinking water studies.
[d]The historical incidence of squamous cell carcinoma in the oral cavity in NCTR control male F344/N Nctr rats is 0.2% (range 0.0–0.6%; Table A-7) for all studies and 0.0% (Table A-7) for drinking water studies.
[e]The historical incidence of squamous cell papilloma, papilloma, or squamous cell carcinoma in the oral cavity in NCTR control male F344/N Nctr rats is 0.5% (range 0.0–2.1%; Table A-7) for all studies and 1.0% (range 0.0–2.1%; Table A-7) for drinking water studies.
[f]The historical incidence of squamous cell papilloma or papilloma in the oral cavity in NCTR control female F344/N Nctr rats is 0.3% (range 0.0–2.1%; Table B-6) for all studies and 0% (Table B-6) for drinking water studies.
[g]The historical incidence of squamous cell carcinoma in the oral cavity in NCTR control female F344/N Nctr rats is 0% (Table B-6) for all studies.
[h]The historical incidence of squamous cell papilloma, papilloma, or squamous cell carcinoma in the oral cavity in NCTR control female F344/N Nctr rats is 0.3% (range 0.0–2.1%; Table B-6) for all studies and 0% (Table B-6) for drinking water studies.

Table 16. Incidences of Mononuclear Cell Leukemia in Rats in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Male[a]					
Overall rate[b]	21/48 (44%)	26/48 (54%)	27/48 (56%)	27/47 (57%)	31/48 (65%)
Poly-3 test[c]	P = 0.008	P = 0.211	P = 0.093	P = 0.097	P = 0.006
Female[d]					
Overall rate	14/48 (29%)	11/48 (23%)	21/48 (44%)	19/48 (40%)	27/48 (56%)
Poly-3 test	<0.001	0.382N	0.076	0.076	<0.001

[a]The historical incidence of mononuclear cell leukemia in NCTR control male F344/N Nctr rats is 47.1% (range 31.3–64.6%; Table A-6) for all studies and 60.4% (range 56.3–64.6%; Table A-6) for drinking water studies.
[b]Number of animals with neoplasm per number of animals examined microscopically.
[c]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.
[d]The historical incidence of mononuclear cell leukemia in NCTR control female F344/N Nctr rats is 33.2% (range 12.5–45.0%; Table B-8) for all studies and 20.8% (range 20.8%; Table B-8) for drinking water studies.

Table 17. Incidences of Malignant Mesothelioma in Male Rats in the Two-year Drinking Water Study of Glycidamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Epididymis					
Overall rate[b]	0/48 (0%)	1/45 (2%)	3/48 (6%)	10/47 (21)%	17/47 (36%)
Poly-3 test[c]	P < 0.001	P = 0.483	P = 0.110	P < 0.001	P < 0.001
Testes					
Overall rate	0/48 (0%)	1/47 (2%)	3/48 (6%)	6/47 (13)%	13/48 (27%)
Poly-3 test	P < 0.001	P = 0.490	P = 0.110	P = 0.010	P < 0.001
Epididymis or Testes					
Overall rate	0/48 (0%)	1/48 (2%)	3/48 (6%)	10/47 (21)%	17/48 (35%)
Poly-3 test	P < 0.001	P = 0.491	P = 0.110	P < 0.001	P < 0.001

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
All Organs					
Overall rate	0/48 (0%)	1/48 (2%)	4/48 (8%)	10/47 (21%)	17/48 (35%)
Poly-3 test	P < 0.001	P = 0.491	P = 0.054	P < 0.001	P < 0.001

[a]The historical incidence of malignant mesothelioma (all sites) in NCTR control male F344/N Nctr rats is 4.5% (range 0.0–6.4%; Table A-4) for all studies and 2.1% (range 0.0–4.2%; Table A-4) for drinking water studies.
[b]Number of animals with neoplasm per number of animals examined microscopically.
[c]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice.

Table 18. Incidences of Neoplasms of the Heart in Male Rats in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Malignant Schwannoma[a]					
Overall rate[b]	2/48 (4%)	3/48 (6%)	3/48 (6%)	7/47 (15%)	8/48 (17%)
Poly-3 test[c]	P = 0.002	P = 0.481	P = 0.478	P = 0.061	P = 0.015

[a]The historical incidence of malignant schwannoma of the heart in NCTR control male F344/N Nctr rats is 0.3% (range 0.0–2.1%; Table A-5) for all studies and 2.1% (range 2.1%; Table A-5) for drinking water studies.
[b]Number of animals with neoplasm per number of animals examined microscopically.
[c]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice.

Table 19. Incidences of Neoplasms of the Mammary Gland in Female Rats in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Fibroadenoma[a]					
Overall rate[b]	16/48 (33%)	26/48 (54%)	35/48 (73%)	33/48 (69%)	36/48 (75%)
Poly-3 test[c]	P < 0.001	P = 0.019	P < 0.001	P < 0.001	P < 0.001

[a]The historical incidence of mammary gland fibroadenoma in NCTR control female F344/N Nctr rats is 34.3% (range 25.5–42.6%; Table B-5) for all studies and 29.5% (range 25.5–33.3%; Table B-5) for drinking water studies.
[b]Number of animals with neoplasm per number of animals examined microscopically.
[c]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice.

Table 20. Incidences of Neoplasms of the Clitoral Gland in Female Rats in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Adenoma					
Overall rate[a]	6/48 (13%)	3/48 (6%)	6/48 (13%)	3/48 (6%)	5/47 (11%)
Poly-3 test[b]	P = 0.340	P = 0.278N	P = 0.570	P = 0.346N	P = 0.409
Carcinoma[c]					
Overall rate	4/48 (8%)	6/48 (13%)	7/48 (15%)	11/48 (23%)	14/47 (30%)
Poly-3 test	P < 0.001	P = 0.345	P = 0.233	P = 0.017	P < .001
Squamous Cell Papilloma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	2/47 (4%)
Poly-3 test	P = 0.013	–	–	P = 0.461	P = 0.136
Squamous Cell Carcinoma					
Overall rate	2/48 (4%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/47 (0%)
Poly-3 test	P = 0.111N	P = 0.252N	P = 0.252N	P = 0.283N	P = 0.387N
Adenoma, Carcinoma, and Squamous Cell Papilloma and Carcinoma (Combined)					
Overall rate	11/48 (23%)	9/48 (19%)	13/48 (27%)	14/48 (29%)	20/47 (43%)
Poly-3 test	P < 0.001	P = 0.442N	P = 0.347	P = 0.165	P = 0.001

[a]Number of animals with neoplasm per number of animals examined microscopically.
[b]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.
[c]The historical incidence of clitoral carcinoma in NCTR control female F344/N Nctr rats is 4.9% (range 0.0–10.4%; Table B-4) for all studies and 4.2% (range 2.1–6.3%; Table B-4) for drinking water studies.

Table 21. Incidences of Neoplasms of the Stomach (Forestomach) in Female Rats in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Squamous Cell Papilloma[a]					
Overall rate[b]	0/48 (0%)	1/48 (2%)	0/48 (0%)	0/47 (0%)	3/46 (7%)
Poly-3 test[c]	P = 0.015	P = 0.487	–	–	P = 0.048

[a]The historical incidence squamous cell papilloma or carcinoma (combined) of the forestomach in NCTR control female F344/N Nctr rats is 0.1% (range 0.0–2.1%; Table B-7) for all studies and 1.0% (range 0.0–2.1%; Table B-7) for drinking water studies.
[b]Number of animals with neoplasm per number of animals examined microscopically.
[c]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice.

Nonneoplastic Findings

Male and female F344/N Nctr rats administered glycidamide in the drinking water had dose-related increases in brain gliosis (Table 22 and Table 23).

Male F344/N Nctr rats also had glycidamide-related increases in exfoliated germ cells in the epididymis, hepatocyte degeneration, and liver necrosis (Table 22). The hepatocellular degeneration was characterized by either affected cells displaying increased cytoplasmic granularity, cell swelling, and eosinophilia or by cystic enlargement of cells of variable size that in some areas contained finely stained eosinophilic material. The hepatocellular necrosis featured single cells with condensed hyper-eosinophilic cytoplasm with an irregular cell outline or by single or multiple foci of swollen hepatocytes with increased eosinophilia and a nucleus undergoing lysis. The distribution of these changes was variable.

Additional nonneoplastic lesions associated with glycidamide exposure in female F344/N Nctr rats included bone marrow hyperplasia, axonal degeneration of the lumbar spinal cord, and uterine endometrial hyperplasia (Table 23).

Table 22. Incidences of Selected Nonneoplastic Lesions in Male Rats in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Brain					
Gliosis					
Overall rate[a]	0/48 (0%)	1/48 (2%)	0/48 (0%)	0/47 (0%)	4/48 (8%)
Poly-3 test[b]	P = 0.003	P = 0.491	–	–	P = 0.030
Average severity[c]	–	1.0	–	–	2.8
Epididymis					
Exfoliated Germ Cells					
Overall rate	0/48 (0%)	1/45 (2%)	2/48 (4%)	3/47 (6%)	4/47 (9%)
Poly-3 test	P = 0.009	P = 0.485	P = 0.228	P = 0.102	P = 0.029
Average severity	–	3.0	2.5	3.0	3.3
Liver					
Hepatocyte Degeneration					
Overall rate	2/47 (4%)	6/47 (13%)	6/48 (13%)	10/47 (21%)	8/47 (17%)
Poly-3 test	P = 0.006	P = 0.128	P = 0.130	P = 0.007	P = 0.011
Average severity	4.0	3.8	3.8	3.6	3.8
Necrosis					
Overall rate	1/47 (2%)	5/47 (11%)	2/48 (4%)	7/47 (15%)	5/47 (11%)
Poly-3 test	P = 0.025	P = 0.092	P = 0.495	P = 0.019	P = 0.043
Average severity	1.0	3.2	3.0	3.0	3.4

[a]Number of animals with lesion per number of animals examined microscopically.
[b]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated (0.0875, 0.175, 0.35, and 0.70 mM glycidamide) group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide

group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice.
[c]Severity was graded as 1, minimal; 2, mild; 3, moderate; and 4, marked.

Table 23. Incidence of Selected Nonneoplastic Lesions in Female Rats in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Brain					
Gliosis					
Overall rate[a]	0/48 (0%)	0/48 (0%)	4/48 (8%)	4/48 (8%)	4/48 (8%)
Poly-3 test[b]	P = 0.003	–	P =0.052	P = 0.042	P = 0.020
Average severity[c]	–	–	2.5	2.0	2.5
Bone Marrow					
Hyperplasia					
Overall rate	2/48 (4%)	6/48 (13%)	7/46 (15%)	8/47 (17%)	14/47 (30%)
Poly-3 test	P < 0.001	P = 0.123	P = 0.067	P = 0.025	P < 0.001
Average severity	2.0	2.3	2.3	2.1	2.4
Spinal Cord (Lumbar)					
Axonal Degeneration					
Overall rate	5/48 (10%)	6/48 (13%)	5/47 (11%)	6/48 (13%)	9/48 (19%)
Poly-3 test	P = 0.027	P = 0.458	P = 0.584	P = 0.388	P = 0.042
Average severity	1.0	1.0	1.0	1.0	1.0
Uterus					
Endometrium, Hyperplasia, Cystic					
Overall rate	11/48 (23%)	17/48 (35%)	14/48 (29%)	14/48 (29%)	23/48 (48%)
Poly-3 test	P < 0.001	P = 0.089	P = 0.277	P = 0.170	P < 0.001
Average severity	1.6	1.5	2.2	2.4	1.8

[a]Number of animals with lesion per number of animals examined microscopically.
[b]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated (0.0875, 0.175, 0.35, and 0.70 mM glycidamide) group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.
[c]Severity was graded as 1, minimal; 2, mild; 3, moderate; and 4, marked.

Mice

Two-week Study

Three of the male mice exposed to 7.03 mM glycidamide in the drinking water displayed an abnormal posture or gait after 14 days of treatment. A single female mouse treated with 7.03 mM glycidamide in the drinking water displayed an abnormal posture and gait after 14 days of treatment. There were no other significant in-life observations in any of the other treatment groups.

Male mice administered 3.52 and 7.03 mM glycidamide in the drinking water for 14 days weighed 87% and 61% of the control mice; female mice given 7.03 mM glycidamide in the drinking water for 14 days weighed 69% of the control mice (Table 24). Body weights in the other treatment groups after 14 days of exposure were within 10% of the control mice.

Water and food consumption were relatively constant at each time point and for each dose group, with the possible exception of food consumption in mice receiving 7.03 mM glycidamide in the drinking water (Table 24). Male mice administered 0.14, 0.35, 0.70, 1.41, 3.52, and 7.03 mM glycidamide in the drinking water consumed approximately 2.8, 6.9, 14.8, 25.4, 77.7, and 167 mg glycidamide per kg body weight per day, respectively; the comparable values for female mice were 3.2, 8.9, 15.1, 32.5, 95.0, and 164 mg glycidamide per kg body weight per day.

There were no nonneoplastic lesions observed either grossly or microscopically that could be attributed to the administration of glycidamide in the drinking water.

Exposure Concentration Selection Rationale: The selection of doses for the 3-month glycidamide drinking water study was based upon the effects observed in the 2-week studies of glycidamide (this study) and acrylamide[4], which were conducted simultaneously. Both 7.03 mM glycidamide and 7.03 mM acrylamide in drinking water resulted in decreased body weight. In addition, none of the mice receiving 7.03 mM acrylamide survived the 2-week treatment period. Since one of the goals of this study was to compare acrylamide with glycidamide, a high dose of 3.52 mM glycidamide (306 ppm glycidamide) was selected for the 3-month drinking water study, with the remaining doses being 0, 0.14, 0.35, 0.70, and 1.41 mM glycidamide (0, 12.2, 30.6, 61.2, and 122 ppm glycidamide). These doses were identical to those used in the 3-month drinking water study with acrylamide[4].

Table 24. Survival, Body Weights, Food Consumption, and Water Consumption of Mice in the Two-week Drinking Water Study of Glycidamide

Treatment	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Mean Food Consumption[c]		Mean Water Consumption[c]	
		Day 1	Day 7	Day 14		Week 1	Week 2	Week 1	Week 2
Drinking Water									
Male									
0.0 mM	4/4	19.0 ± 0.7	20.9 ± 0.9	22.1 ± 1.0		2.8 (100)	3.2 (100)	5.0 (100)	4.7 (100)
0.14 mM	4/4	19.0 ± 0.6	20.7 ± 0.7	20.0 ± 0.6	91	2.6 (93)	3.0 (94)	5.1 (102)	4.3 (91)
0.35 mM	4/4	18.9 ± 0.9	20.9 ± 0.6	21.4 ± 0.6	97	2.9 (104)	3.2 (100)	5.3 (106)	4.3 (91)
0.70 mM	4/4	19.1 ± 0.7[d]	20.3 ± 1.0[d]	21.4 ± 0.5[d]	97	4.2 (150)	3.1 (97)	5.6 (112)	4.5 (96)
1.41 mM	4/4	19.2 ± 0.4	20.4 ± 0.5	20.7 ± 0.6	94	2.7 (96)	3.2 (100)	3.3 (66)	5.2 (111)
3.52 mM	4/4	19.1 ± 0.8	19.0 ± 0.6	19.3 ± 0.8*	87	3.0 (107)	2.9 (91)	5.2 (104)	4.5 (96)
7.03 mM	4/4	19.6 ± 0.7	15.7 ± 0.6*	13.4 ± 0.4*	61	1.8 (64)	1.6 (50)	4.6 (92)	3.4 (72)
Female									
0.0 mM	4/4	15.4 ± 0.3	16.5 ± 0.05	16.3 ± 0.2		2.4 (100)	3.6 (100)	5.7 (100)	4.2 (100)
0.14 mM	4/4	15.6 ± 0.4	17.4 ± 0.2	16.9 ± 0.1	104	2.7 (113)	2.8 (78)	5.0 (88)	4.1 (98)
0.35 mM	4/4	15.2 ± 0.1	16.7 ± 0.3	16.5 ± 0.2	102	2.9 (121)	2.7 (75)	5.3 (93)	4.4 (105)
0.70 mM	4/4	15.1 ± 0.6	16.1 ± 0.5	16.7 ± 0.3	103	2.8 (117)	3.0 (83)	4.3 (75)	3.8 (90)
1.41 mM	4/4	15.5 ± 0.3	16.6 ± 0.2	16.3 ± 0.1	100	2.4 (100)	2.6 (72)	4.7 (82)	4.0 (95)
3.52 mM	4/4	15.4 ± 0.3	14.8 ± 0.5*	14.9 ± 0.5*	92	2.2 (92)	2.3 (64)	5.1 (89)	4.1 (98)
7.03 mM	4/4	15.9 ± 0.3	13.4 ± 0.1*	11.3 ± 0.3*	69	1.5 (63)	1.8 (50)	4.1 (72)	2.6 (62)

[a]Number of animals surviving at 14 days/number initially in group.
[b]Weights are given as mean ± standard error. An asterisk (*) denotes significant difference ($p < 0.05$) from control at the same time point.
[c]Food and water consumption are expressed as grams per animal per day and were measured on a per cage basis. Values in parentheses indicate the percentage of controls. Statistical analyses were not conducted on food and water consumption because there was only one cage per treatment group.
[d]Based upon three animals: at necropsy, one of the mice was discovered to be mis-sexed.

Three-month Study

One female mouse administered 1.41 mM glycidamide in the drinking water died on day 22 (Table 25). Two male mice administered 3.52 mM glycidamide in the drinking water developed hind limb paresis beginning at 68 to 70 days on the experiment. The paresis was not of sufficient severity to necessitate removing the mice from the study. Hind limb paresis did not occur in female mice administered 3.52 mM glycidamide. There were no other significant in-life observations in any of the other treatment groups.

Glycidamide in the drinking water resulted in significant dose-related effects on body weight in male mice only (Table 25 and Figure 9). Pairwise comparisons indicated that 3.52 mM glycidamide was associated with significant decreases in body weight gain in the male mice over the 3 month treatment period. At the end of the 3-month period, the mice weighed 90% of the male control group.

Table 25. Survival and Body Weights of Mice in the Three-month Drinking Water Study of Glycidamide

Treatment	Survival[a]	Mean Body Weight (g)[b]		Final weight Relative to Controls (%)
		Week 0	Week 14	
Male				
0.0 mM	8/8	17.9 ± 0.4	27.1 ± 0.4	
0.14 mM	8/8	18.1 ± 0.4	27.2 ± 0.4	100
0.35 mM	8/8	16.7 ± 0.4	29.5 ± 0.4	109
0.70 mM	8/8	17.8 ± 0.4	25.4 ± 0.4	94
1.41 mM	8/8	17.4 ± 0.4	25.1 ± 0.4	92
3.52 mM	8/8	18.1 ± 0.4	24.4 ± 0.4	90
Female				
0.0 mM	8/8	13.9 ± 0.2	21.3 ± 0.2	
0.14 mM	8/8	13.6 ± 0.2	21.1 ± 0.2	99
0.35 mM	8/8	13.5 ± 0.2	21.5 ± 0.2	101
0.70 mM	8/8	14.6 ± 0.2	21.5 ± 0.2	101
1.41 mM	7/8	14.4 ± 0.2	20.9 ± 0.2	98
3.52 mM	8/8	14.6 ± 0.2	19.5 ± 0.2	91

[a]Number of animal surviving until study termination/number of animals initially in group.
[b]Weights are given as LS means ± population standard error of the mean.

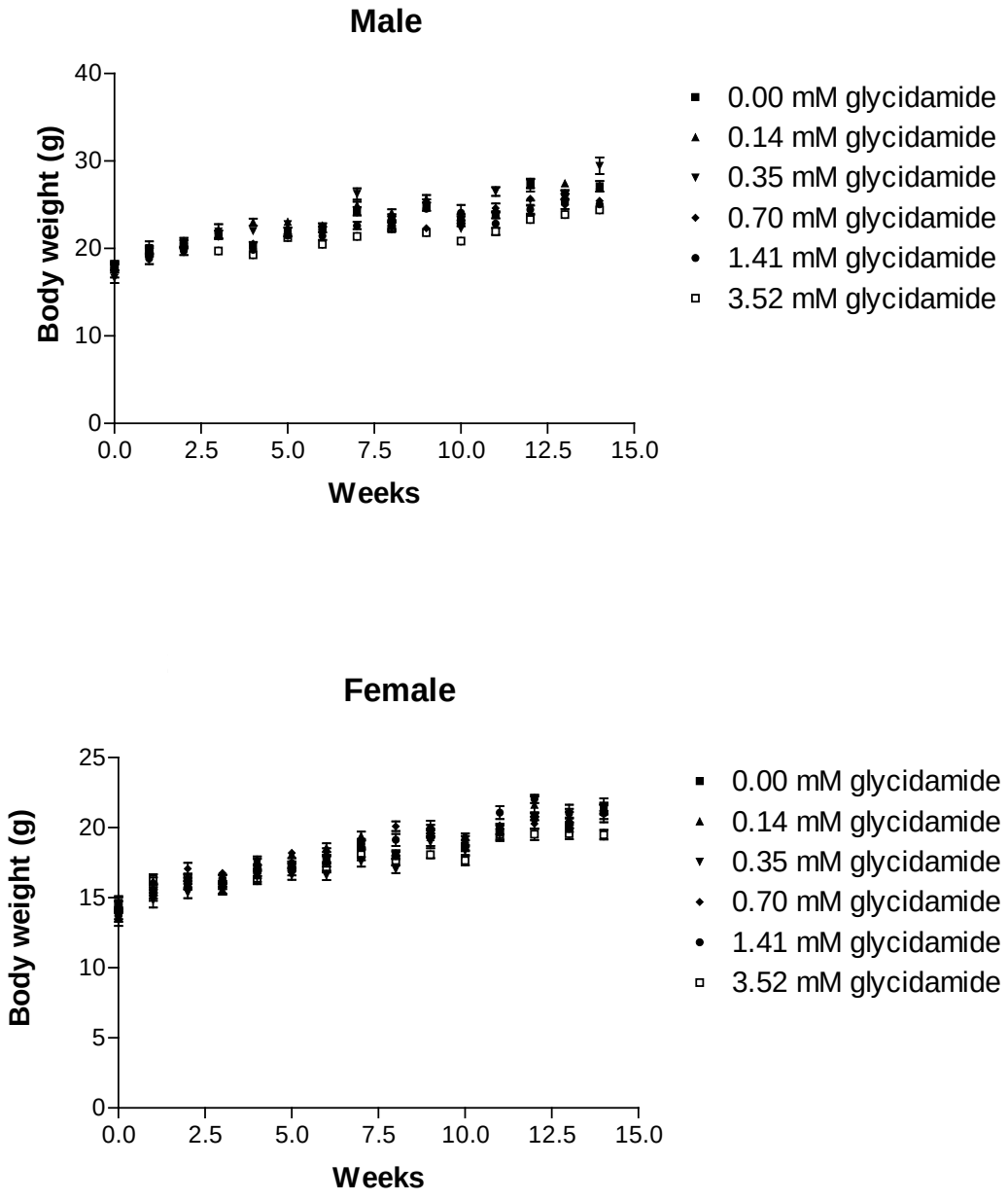


Figure 9. Growth Curves for Male and Female Mice in the Three-month Drinking Water Study of Glycidamide

Male mice given 0.0, 0.14, 0.35, 0.70, 1.41, and 3.52 mM glycidamide in the drinking water consumed approximately 6.4, 6.1, 7.0, 7.0, 6.5, and 5.7 ml drinking water per day (Table 26), which was equivalent to 0.0, 3.2, 9.1, 19.2, 36.0, and 81.5 mg glycidamide per kg body weight per day; the comparable values for female mice were 6.4, 6.2, 6.3, 6.1, 6.8, and 5.5 ml drinking water per day (Table 26), which was equivalent to 0.0, 4.1, 10.8, 20.1, 45.3, and 96.5 mg glycidamide per kg body weight per day.

Male mice given 0.0, 0.14, 0.35, 0.70, 1.41, and 3.52 mM glycidamide in the drinking water consumed approximately 3.4, 4.1, 3.5, 3.3, 3.2, and 3.2 g feed per day (Table 27); the comparable values for female mice were 4.2, 4.0, 3.8, 3.7, 4.0, and 3.7 g feed per day (Table 27).

Necropsy body weights were decreased in male and female mice administered 3.52 mM glycidamide in the drinking water for 3 months (Table E-4). Necropsy body weights and the liver-weight-to-brain-weight ratio were increased in male mice receiving 0.35 mM glycidamide. Brain weights were decreased in male mice given 3.52 mM glycidamide (Table E-4).

The only gross observation in the mice given glycidamide in the drinking water that was considered to be treatment related was marked dilatation of the urinary bladder in one of eight males in the 3.52 mM group (Table 28). This animal also had a clinical observation of partial paresis of the rear legs.

Target tissues (sciatic nerve, spinal cord, skeletal muscle of the hind limb, testes, and ovaries) were examined microscopically in progressively lower dose groups until a no-observed-adverse-effect level was reached. Peripheral neuropathy was observed in the sciatic nerve of one female and one male treated with 3.52 mM glycidamide (Table 28). The changes were characterized by nerve fiber degeneration with dilatation and vacuolization of myelin sheaths along with swollen and shrunken axons, with minimal severity. In male mice, minimal to mild depletion of the testicular germinal cell epithelium occurred in seven of eight mice administered 3.52 mM glycidamide (Table 28).

Exposure Concentration Selection Rationale: The selection of doses for the 2-year glycidamide drinking water study was based upon the effects observed in the 3-month drinking water studies with glycidamide (this study) and acrylamide[4], which were conducted simultaneously. Both 3.52 mM glycidamide and 3.52 mM acrylamide in drinking water resulted in hind limb paresis and decreased body weight. Decreases in body weight were also observed with 1.41 mM acrylamide. Since one of the goals of this study was to compare acrylamide with glycidamide, a high dose of 0.70 mM glycidamide (61.2 ppm glycidamide) was selected for the chronic 2-year drinking water study, with the remaining doses being 0.0, 0.0875, 0.175, and 0.35 mM glycidamide (0, 7.65, 15.3, and 30.6 ppm glycidamide). These doses were identical to those used in the 2-year chronic bioassay with acrylamide[4].

Table 26. Water Consumption[a] of Mice in the Three-month Drinking Water Study of Glycidamide

Week	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM
Male						
2	4.9	4.9	5.0	4.7	5.7	5.0
3	7.0	8.7	6.9	7.9	6.8	8.7
4	5.6	6.6	6.7	11.8	5.8	5.1
5	5.4	6.1	6.1	5.1	5.3	5.0
6	6.9	6.8	7.3	7.3	7.2	6.7
7	8.0	7.4	8.0	9.6	7.4	5.6
8	6.6	6.0	6.3	7.0	5.8	5.2
9	6.4	6.3	6.5	6.3	6.8	5.5
10	5.9	6.6	6.2	7.5	7.3	5.4
11	4.7	3.1	7.9	7.8	6.6	5.6
12	7.3	6.1	9.7	6.1	7.2	5.5
13	9.9	6.1	7.5	6.0	6.3	5.4
14	5.3	5.4	7.1	5.0	7.1	5.1
Female						
2	3.9	5.7	3.6	5.0	6.0	4.1
3	5.9	7.0	6.0	6.7	7.6	5.6
4	6.3	5.4	6.1	6.0	6.7	5.1
5	5.2	5.1	5.0	4.1	5.9	4.1
6	7.6	5.8	5.1	5.9	7.5	5.5
7	6.2	5.2	6.0	5.7	5.7	4.6
8	5.4	6.3	5.9	5.7	6.7	4.4
9	5.4	5.4	5.1	4.6	5.4	4.0
10	5.5	4.9	6.6	4.9	5.4	4.8
11	4.9	4.4	6.0	7.0	5.4	8.0
12	5.0	4.9	4.6	4.8	5.4	4.0
13	5.9	4.9	6.4	5.2	5.8	5.0

[a]Water consumption is given the mean of two cages and is expressed as grams per animal per day.

Table 27. Food Consumption[a] of Mice in the Three-month Drinking Water Study of Glycidamide

Week	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM
Male						
3	4.7	6.2	4.0	3.4	3.8	5.8
4	2.5	3.6	3.1	2.7	2.8	2.7
5	3.1	3.3	3.7	3.6	3.5	3.9
6	3.3	3.1	2.6	3.1	3.1	2.8
7	3.5	4.1	4.4	3.3	3.4	3.1
8	2.7	3.0	2.9	3.1	3.3	3.0
9	3.8	4.5	3.4	3.2	2.8	2.7
10	2.8	3.3	2.7	3.0	3.0	2.5
11	1.0	7.0	4.3	4.1	1.4	1.1
12	7.3	8.1	3.4	3.6	5.1	4.0
13	2.8	3.4	4.1	3.0	3.5	3.0
14	3.2	3.1	3.2	3.1	2.8	3.5
Female						
3	4.1	6.9	2.8	2.8	4.6	3.4
4	2.7	3.7	3.7	3.2	2.9	2.7
5	3.7	3.4	2.9	2.8	3.1	2.9
6	6.0	3.0	1.9	3.0	3.1	2.8
7	3.2	3.4	4.0	3.4	2.7	3.1
8	2.7	2.6	2.7	4.2	3.9	2.8
9	3.4	3.4	3.4	3.1	3.6	3.2
10	2.6	2.6	3.9	2.8	3.0	3.0
11	5.2	1.2	1.9	2.0	2.2	5.6
12	4.4	6.5	3.5	3.7	7.4	3.4
13	2.9	2.6	3.6	2.9	3.0	2.9

[a]Food consumption is given the mean of two cages and is expressed as grams per animal per day.

Table 28. Incidence of Observations and Nonneoplastic Lesions in Mice in the Three-month Drinking Water Study of Glycidamide[a]

	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM
Males						
Animals initially in study	8	8	8	8	8	8
Hind limb						
Paresis	0	0	0	0	0	2
Peripheral nerve						
Axon degeneration	0	–[b]	–	–	–	1 (1.0)
Urinary bladder						
Dilatation	0	–	–	–	–	1 (4.0)
Testes						
Germinal epithelium degeneration	0	–	–	–	0	7 (1.7)
Females						
Animals initially in study	8	8	8	8	8	8
Hind limb						
Paresis	0	0	0	0	0	0
Peripheral nerve						
Axon degeneration	0	–	–	–	–	1 (1.0)
Urinary bladder						
Dilatation	0	–	–	–	–	0

[a]Data are reported as the number of lesions per number of mice (8) examined microscopically. The average severity is given in parentheses. Severity was scored as: 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.
[b]Not examined.

Two-year Study

Survival and Cause of Death

Glycidamide in the drinking water resulted in a dose-related trend in survival in male and female B6C3F1/Nctr mice (Table 29 and Figure 10). Compared to control mice, male mice administered 0.175, 0.35, and 0.70 mM glycidamide and female mice administered 0.35 and 0.70 mM glycidamide had decreased survival. The primary cause for early removal or death of male mice was neoplasms, including Harderian gland adenoma, malignant lymphoma, hepatocellular carcinoma, and various types of mesenchymal skin tumors. The primary cause for early removal or death of female mice was neoplasms, including Harderian gland adenoma, malignant lymphoma, mammary gland adenoacanthoma or adenocarcinoma, and various types of mesenchymal skin tumors.

Table 29. Survival and Disposition of Mice in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Male					
Animals initially in study	48	48	48	48	48
Moribund	2	4	12	18	13
Natural deaths	1	3	2	4	10
Animals surviving to study termination[a]	45	41	34	26	25
Percent probability of survival at end of study[b]	94	85	71	54	52
Mean survival (weeks)[c]	100.5	97.9	97.1	94.7	93.9
Survival analysis[d]	P < 0.001	P = 0.191	P = 0.008	P < 0.001	P < 0.001
Female					
Animals initially in study	48	48	48	48	48
Moribund	4	2	8	12	34
Natural deaths	3	4	2	5	6
Animals surviving to study termination	41	42	38	31	8
Percent probability of survival at end of study	85	88	79	67	17
Mean survival (weeks)	101.1	98.9	100.5	98.3	82.6
Survival analysis	P < 0.001	P = 0.804	P = 0.408	P = 0.024	P < 0.001

[a]Censored from the survival analyses.
[b]Kaplan-Meier survival estimates.
[c]Mean of all deaths (censored and uncensored).
[d]The result of the trend test is in the 0.0 mM glycidamide column, and the results of the pairwise comparisons[62] with the 0.0 mM glycidamide are in the treatment group columns.

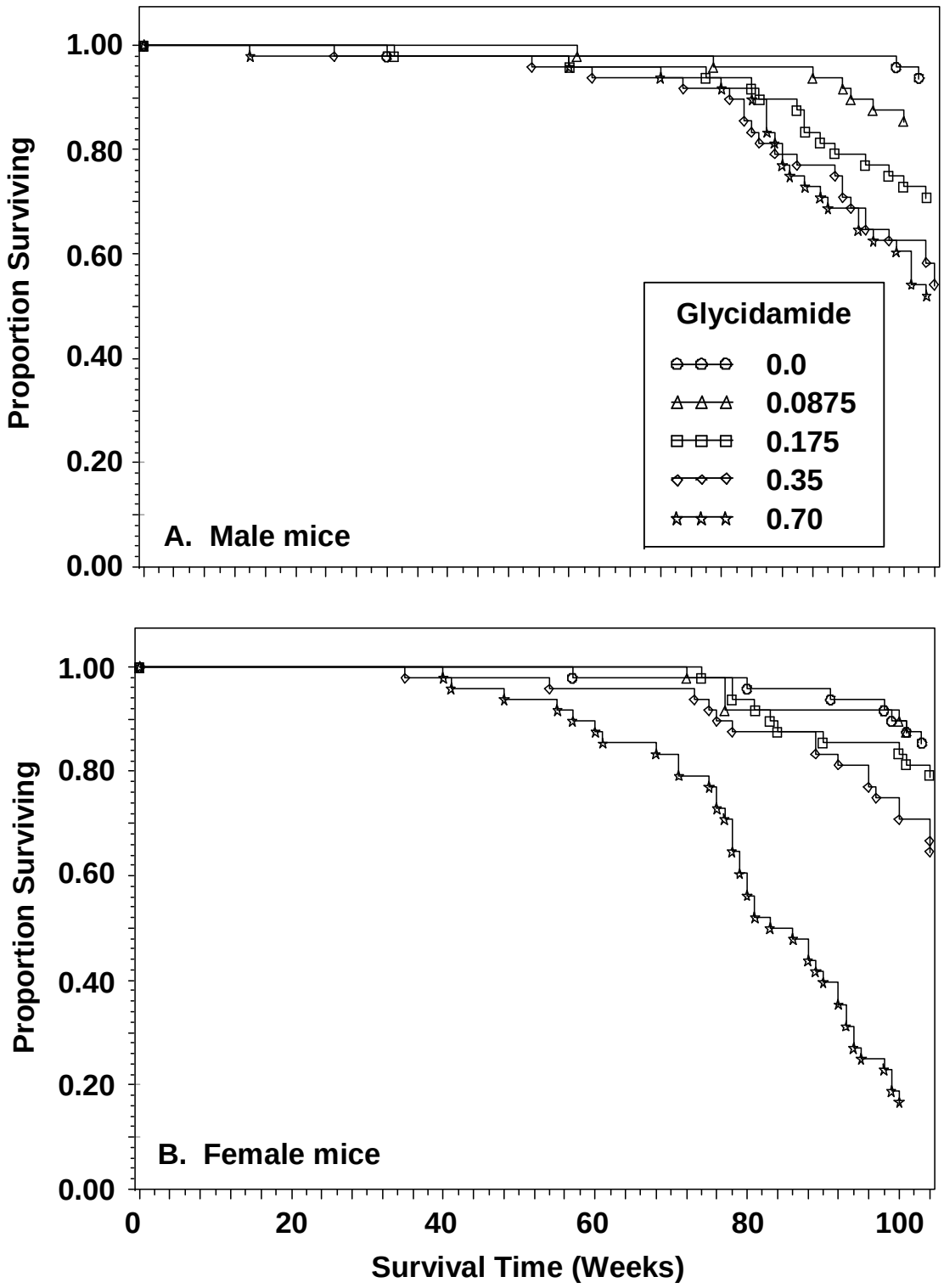


Figure 10. Kaplan-Meier Survival Curves for Male and Female Mice Administered Glycidamide (mM) in Drinking Water for Two Years

Body Weights and Food and Water Consumption

There were no significant body weight changes in male B6C3F1/Nctr mice administered glycidamide in the drinking water (Figure 11 and Table 30). Administering glycidamide in the drinking water to female B6C3F1/Nctr mice resulted in only sporadic statistically significant decreases in body weight, with the magnitude of the change never exceeding 5% of the mean control body weight at the same time point (Figure 11 and Table 31).

Glycidamide in the drinking water resulted in dose-related trends in food consumption in male B6C3F1/Nctr mice beginning at week 84 (Table G-3) and in female B6C3F1/Nctr mice beginning at week 60 (Table G-4). In male mice, pairwise comparisons indicated that food consumption was significantly increased at later time points in the 0.35 and 0.70 mM glycidamide groups (Table G-3). In female mice, food consumption in the 0.70 mM glycidamide group was significantly increased at weeks 68 and 76 to 104 (Table G-4) compared to the control group.

Glycidamide in the drinking water resulted in sporadic dose-related trends in water consumption in male B6C3F1/Nctr mice (Table 32). In female mice, there were dose-related trends in water consumption beginning at week 76 (Table 33). Pairwise comparisons indicated that water consumption in the 0.70 mM glycidamide group of female mice was significantly increased compared to the control group beginning at week 80 (Table 33).

The mean glycidamide exposure for mice, calculated at 4 week intervals, is presented in Table 32 and Table 33 and Figure 12. The mean amount of glycidamide consumed by male mice for the entire 2-year experiment was 1.20, 2.65, 5.13, and 9.55 mg glycidamide per kg body weight per day for the 0.0875, 0.175, 0.35, and 0.70 mM glycidamide dose groups, respectively (Table 32 and Figure 12). The corresponding values for female mice were 1.37, 2.89, 5.64, and 12.99 mg glycidamide per kg body weight per day (Table 33 and Figure 12).

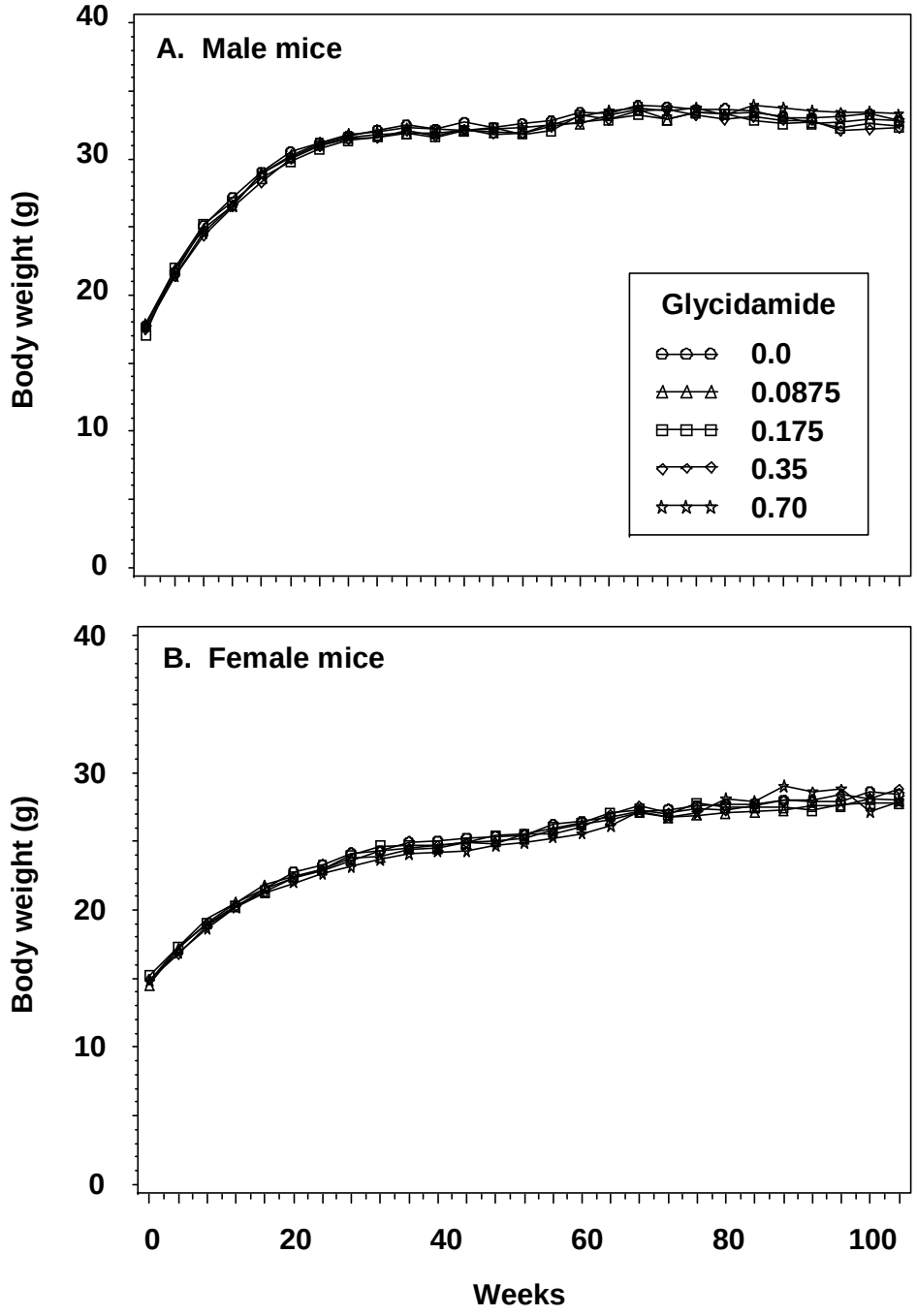


Figure 11. Growth Curves for Male and Female Mice Administered Glycidamide (mM) in Drinking Water for Two Years

Table 30. Mean Body Weights and Survival of Male Mice in the Two-year Drinking Water Study of Glycidamide

Weeks on Study	0 mM		0.0875 mM			0.175 mM			0.35 mM			0.70 mM		
	Mean Wt. (g)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors
4	21.7	48	21.9	101.0	48	22.0	101.6	48	21.5	98.9	48	21.4	98.7	48
8	25.1	48	25.0	99.3	48	25.2	100.2	48	24.4	97.0	48	24.6	98.0	48
12	27.2	48	26.6	97.8	48	26.8	98.6	48	26.5	97.5	48	26.6	97.7	48
16	29.0	48	29.0	99.8	48	28.6	98.5	48	28.3	97.4	48	28.9	99.4	47
20	30.5	48	30.2	98.7	48	29.9	97.8	48	30.1	98.4	48	30.2	98.9	47
24	31.2	48	31.1	99.5	48	30.8	98.7	48	31.0	99.2	48	31.2	99.8	47
28	31.7	48	31.6	99.6	48	31.4	98.9	48	31.5	99.4	47	31.8	100.2	47
32	32.1	48	31.8	98.8	48	31.6	98.4	48	31.6	98.4	47	32.0	99.5	47
36	32.5	47	31.9	98.2	48	31.8	98.0	47	32.1	98.7	47	32.3	99.3	47
40	32.2	47	31.9	99.1	48	31.6	98.2	47	31.7	98.3	47	32.2	99.9	47
44	32.7	47	32.1	98.2	48	32.1	98.2	47	32.2	98.7	47	32.1	98.3	47
48	32.3	47	32.0	98.9	48	32.3	99.9	47	31.9	98.5	47	32.2	99.6	47
52	32.6	47	32.0	98.1	48	31.8	97.7	47	31.9	97.9	46	32.3	99.2	47
56	32.8	47	32.7	99.5	48	32.1	97.9	47	32.4	98.8	46	32.5	99.1	47
60	33.4	47	32.6	97.7	47	33.3	99.6	46	32.7	98.0	45	33.0	98.9	46
64	33.4	47	33.3	99.8	47	33.0	98.9	46	32.9	98.7	45	33.5	100.3	46
68	33.9	47	33.7	99.3	47	33.3	98.2	46	33.6	99.0	45	33.7	99.4	46
72	33.8	47	32.9	97.3	47	33.0	97.5	46	33.7	99.6	44	33.5	99.1	45
76	33.6	47	33.5	99.5	46	33.5	99.5	45	33.2	98.8	44	33.7	100.3	45
80	33.7	47	33.3	98.8	46	33.4	99.0	45	32.8	97.4	41	33.1	98.3	43
84	33.6	47	33.4	99.7	46	33.0	98.5	43	33.0	98.3	38	33.7	100.5	39
88	33.1	47	33.2	100.3	46	32.8	99.2	40	32.8	99.0	37	33.4	100.9	35
92	33.0	47	32.7	98.9	45	32.9	99.7	38	32.8	99.4	36	33.2	100.4	33
96	33.1	47	32.6	98.5	43	32.5	98.1	37	32.4	97.7	31	33.1	100.1	31
100	33.3	46	32.7	98.4	42	32.8	98.7	36	32.1	96.7	30	33.1	99.6	29
104	32.9	45	32.6	99.3	41	32.6	99.3	34	32.3	98.3	28	33.0	100.5	25
Mean for Weeks														
4–104	31.7		31.4			31.3			31.2			31.6		

Table 31. Mean Body Weights and Survival of Female Mice in the Two-year Drinking Water Study of Glycidamide

Weeks on Study	0 mM		0.0875 mM			0.175 mM			0.35 mM			0.70 mM		
	Mean Wt. (g)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors	Mean Wt. (g)	Wt. (% of controls)	No. of Survivors
4	17.3*	48	17.2	99.6	48	17.3	99.9	48	16.8	97.1	48	16.9	97.6	48
8	19.3*	48	19.0	98.5	48	19.0	98.3	48	18.9	97.7	48	18.7*	96.6	48
12	20.5	48	20.6	100.4	48	20.3	99.2	48	20.3	98.8	48	20.2	98.5	48
16	21.6	48	21.8	101.3	48	21.4	99.0	48	21.6	100.1	48	21.3	98.6	48
20	22.8*	48	22.4	98.4	48	22.4	98.2	48	22.3	98.0	48	22.0*	96.5	48
24	23.3*	48	23.0	98.5	48	23.0	98.5	48	22.9	98.3	48	22.7	97.2	48
28	24.1*	48	23.8	98.4	48	24.0	99.2	48	23.6	97.9	48	23.2*	96.0	48
32	24.3	48	23.9	98.2	48	24.7	101.4	48	24.3	99.8	48	23.7	97.5	48
36	25.0*	48	24.4	97.9	48	24.7	99.0	48	24.6	98.3	47	24.1*	96.4	48
40	25.0*	48	24.5	97.8	48	24.7	98.8	48	24.7	98.8	47	24.3*	96.9	48
44	25.3*	48	24.9	98.6	48	24.9	98.6	48	24.9	98.6	47	24.3*	96.3	46
48	25.4*	48	25.0	98.5	48	25.4	100.1	48	24.9	98.0	47	24.7	97.4	46
52	25.5	48	25.2	99.0	48	25.5	100.1	48	25.5	100.1	47	24.9	97.7	45
56	26.2*	48	25.8	98.4	48	26.0	99.1	48	25.5	97.3	46	25.3*	96.5	44
60	26.4*	47	26.3	99.5	48	26.3	99.8	48	26.0	98.6	46	25.6*	96.9	43
64	26.7	47	26.6	99.3	48	27.1	101.2	48	27.0	100.9	46	26.2	97.8	41
68	27.1	47	27.1	100.0	48	27.3	100.7	48	27.6	101.7	46	27.2	100.2	41
72	27.3	47	26.8	98.0	48	27.0	98.8	48	27.1	99.1	46	26.9	98.4	38
76	27.6	47	26.9	97.5	47	27.8	100.7	47	27.4	99.4	44	27.2	98.5	37
80	27.7	47	27.0	97.5	44	27.5	99.5	45	27.3	98.7	42	28.0	101.3	29
84	27.9	46	27.1	97.3	44	27.5	98.8	43	27.6	99.0	42	28.0	100.3	24
88	28.1*	46	27.3	97.0	44	27.6	98.4	42	28.0	99.7	42	29.2	103.8	23
92	27.9*	45	27.6	98.8	44	27.5	98.6	41	28.1	100.5	40	28.9	103.5	19
96	28.0*	45	27.6	98.7	44	27.9	99.7	41	28.5	101.7	39	29.2	104.5	12
100	28.7	43	28.1	98.1	44	27.9	97.3	41	28.2	98.5	36	27.9	97.2	9
104	28.6	41	28.1	98.3	42	28.0	97.9	39	28.9	101.1	34	28.0	97.8	8
Mean for Weeks														
4–104	25.3		24.9			25.1			25.1			24.9		

In the 0.0 mM glycidamide column "" indicates a significant trend ($p < 0.05$); in the treatment column "*" indicates a significant ($p < 0.05$) pairwise comparison of the dose group to the 0.0 mM glycidamide group as determined by Dunnett's test.

Table 32. Water and Glycidamide Consumption by Male Mice in the Two-year Drinking Water Study of Glycidamide

Week[a]	0 mM	0.0875 mM		0.175 mM		0.35 mM		0.70 mM	
	Water (g/day)	Water (g/day)	Dose[b]	Water (g/day)	Dose	Water (g/day)	Dose	Water (g/day)	Dose
4	4.9	4.8	1.78	5.2	3.81	4.9	7.36	5.0	14.75
8	5.0	4.9	1.57	5.3	3.40	4.8	6.35	4.9	12.63
12	5.2	5.1	1.51	5.4	3.14	5.0	5.97	5.0	11.80
16	5.6	5.6	1.52	5.6	3.09	5.3	5.85	5.2	11.53
20	5.8	5.7	1.47	5.7	2.97	5.3	5.54	5.4	11.28
24	5.4*	5.3	1.33	5.4	2.72	5.2	5.20	4.9	9.77
28	5.1	5.2	1.26	5.2	2.58	5.0	4.92	4.8	9.43
32	5.0*	5.1	1.22	5.2	2.49	5.0	4.78	4.7	9.04
36	5.1	5.1	1.22	5.4	2.61	5.0	4.73	4.7	8.95
40	4.8*	4.6	1.11	5.1	2.48	4.8	4.57	4.5	8.47
44	4.8*	4.7	1.12	5.1	2.43	4.8	4.61	4.5	8.54
48	4.8	4.9	1.17	5.1	2.43	4.7	4.49	4.5	8.64
52	4.6*	4.4	1.04	4.8	2.27	4.6	4.40	4.2	7.82
56	4.6	4.5	1.07	4.9	2.36	4.7	4.43	4.4	8.27
60	4.5	4.4	1.03	5.1	2.37	4.6	4.31	4.4	8.17
64	4.8	4.6	1.05	4.8	2.23	4.6	4.31	4.4	8.13
68	5.2	4.4*	1.01	5.0	2.28	4.5	4.16	4.5	8.17
72	4.8	4.6	1.07	5.4	2.47	4.9	4.47	4.5	8.30
76	5.0	4.8	1.11	5.2	2.41	4.8	4.44	4.6	8.30
80	4.8	4.8	1.09	5.3	2.47	5.5*	5.30	4.6	8.60
84	4.8	4.5	1.05	5.1	2.38	5.5	5.11	4.6	8.51
88	4.6*	4.5	1.04	5.3	2.43	5.4	5.07	5.5	9.80
92	4.7	4.9	1.14	5.6	2.62	5.3	5.01	5.3	9.76
96	4.6*	4.6	1.06	5.5	2.57	6.1*	6.04	5.3	9.73
100	5.1	4.7	1.09	6.3	2.94	6.4	6.03	5.4	9.94
104	4.3	4.6	1.06	6.1*	2.90	6.2*	5.90	5.3	9.96
Mean for Weeks									
4–104	4.9	4.8	1.20	5.3	2.65	5.1	5.13	4.8	9.55

[a]Week indicates the last week of a 4-week interval of daily water consumption, measured weekly by cage.
[b]Dose is expressed as the mean value measured in mg/kg body weight/day.
In the 0.0 mM glycidamide column "" indicates a significant trend ($p < 0.05$); in the treatment column "*" indicates a significant ($p < 0.05$) pairwise comparison of the dose group to the 0.0 mM glycidamide group as determined by Dunnett's test.

Table 33. Water and Glycidamide Consumption by Female Mice in the Two-year Drinking Water Study of Glycidamide

Week[a]	0 mM	0.0875 mM		0.175 mM		0.35 mM		0.70 mM	
	Water (g/day)	Water (g/day)	Dose[b]	Water (g/day)	Dose	Water (g/day)	Dose	Water (g/day)	Dose
4	4.4	4.5	2.09	4.5	4.12	4.4	8.34	4.7	19.54
8	4.4	4.5	1.84	4.3	3.54	4.2	7.08	4.2	14.25
12	4.3	4.3	1.65	4.3	3.32	4.2	6.48	4.1	12.90
16	4.1	4.3	1.54	4.3	3.12	4.3	6.23	4.2	12.21
20	4.2	4.1	1.43	4.1	2.85	4.1	5.71	4.3	12.26
24	4.0	4.1	1.37	4.0	2.67	4.1	5.48	4.0	10.84
28	4.2	4.2	1.38	4.1	2.68	4.1	5.34	4.1	10.94
32	4.1	4.1	1.32	4.2	2.60	4.3	5.40	4.2	10.77
36	4.3	4.3	1.34	4.4	2.73	4.3	5.46	4.3	10.94
40	4.4	4.2	1.30	4.3	2.67	4.4	5.49	4.2	10.64
44	4.2	4.1	1.27	4.5	2.75	4.4	5.44	4.2	10.61
48	4.5	4.1	1.26	4.5	2.77	4.3	5.27	4.4	11.00
52	4.3	4.1	1.23	4.3	2.54	4.1	4.97	4.4	10.70
56	4.3	4.1	1.22	4.4	2.60	4.6	5.49	4.4	10.76
60	4.4	4.2	1.23	4.7	3.07	4.5	5.22	4.4	10.62
64	4.5	4.2	1.20	4.3	2.48	4.4	5.03	4.6	10.67
68	4.7	4.2	1.18	4.5	2.51	4.3	4.87	4.6	10.46
72	4.3	4.2	1.20	5.1	2.85	4.6	5.10	5.3	12.06
76	4.6*	4.7	1.34	5.0	2.80	4.8	5.36	5.7	12.83
80	4.7*	4.6	1.32	4.7	2.59	4.7	5.29	5.8*	13.42
84	4.4*	4.8	1.33	4.8	2.72	4.9	5.43	6.4*	14.09
88	4.6*	4.9	1.35	5.0	2.77	5.0	5.47	7.4*	15.72
92	4.5*	4.8	1.33	5.9	3.28	5.0	5.51	7.4*	16.26
96	4.7*	4.7	1.30	5.7	3.19	4.9	5.41	8.9*	19.89
100	5.0*	4.8	1.33	5.7	3.20	5.3	5.76	8.5*	18.45
104	5.1*	4.7	1.28	5.0	2.76	5.3	5.88	9.8*	20.15
Mean for Weeks									
4–104	4.4	4.4	1.37	4.6	2.89	4.5	5.64	5.3	12.99

[a]Week indicates the last week of a 4-week interval of daily water consumption, measured weekly by cage.
[b]Dose is expressed as the mean value measured in mg/kg body weight/day.
In the 0.0 mM glycidamide column "" indicates a significant trend ($p < 0.05$); in the treatment column "*" indicates a significant ($p < 0.05$) pairwise comparison of the dose group to the 0.0 mM glycidamide group as determined by Dunnett's test.

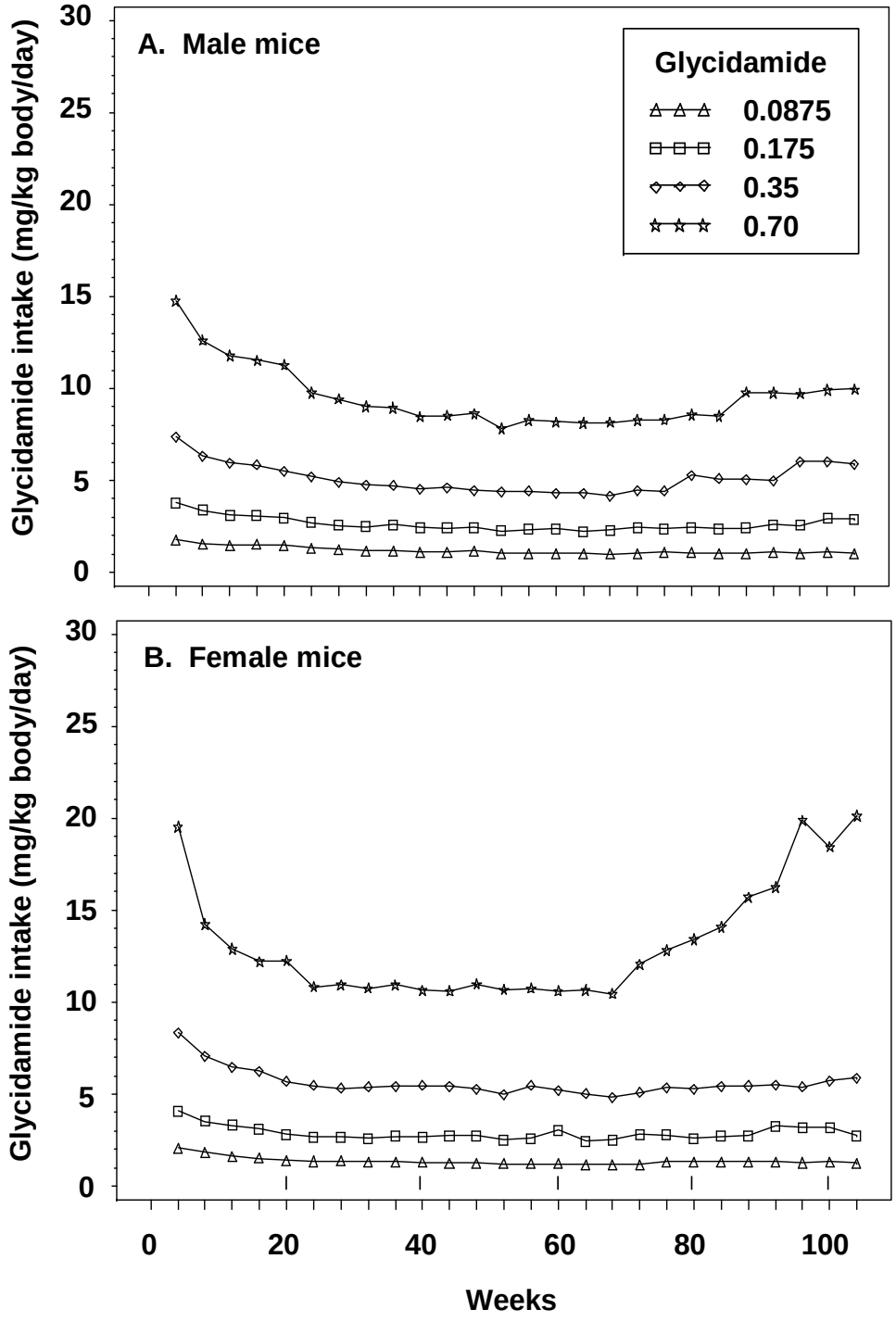


Figure 12. Glycidamide Intake in Male and Female Mice Administered Glycidamide (mM) in Drinking Water for Two Years

Neoplastic Findings

Glycidamide in the drinking water resulted in significant dose-related trends in the incidence of Harderian gland adenoma in both sexes of B6C3F1/Nctr mice (Table 34). Compared to the control groups the incidence of Harderian gland adenoma was significantly increased at all doses of glycidamide. The Harderian gland adenomas were characterized by cuboidal-to-tall columnar neoplastic cells that generally had an abundant foamy pale cytoplasm with round-to-ovoid nuclei. Several patterns were present, including papillary, cystic, and cystic papillary. Harderian gland carcinoma was present in a single female mouse administered 0.175 mM glycidamide and in a single male mouse administered 0.70 mM glycidamide.

A dose-related increase in alveolar/bronchiolar adenoma of the lung was observed in both sexes of B6C3F1/Nctr mice (Table 35). The incidence of alveolar/bronchiolar adenoma was significantly increased at all doses of glycidamide in male mice and at 0.70 mM glycidamide in female mice. A low incidence (≤5%) of alveolar/bronchiolar carcinoma was observed in both sexes of mice, but this did not appear to be related to glycidamide treatment. The alveolar/bronchiolar adenomas were primarily of the papillary type, with tumor cells supported by a fine fibrovascular stroma forming short projections that extended into the alveolar sacs. Tumor margins were well demarcated and compression of the surrounding tissue was distinct. Other types of growth patterns, such as solid or mixed, were present, but with a lower incidence. Carcinomas were irregular growths displaying a pleomorphic histologic pattern, with some being highly infiltrative and poorly demarcated.

Dose-related increases in squamous cell papilloma of the forestomach occurred in male and female B6C3F1/Nctr mice, with the incidence being increased significantly in the 0.70 mM glycidamide group (Table 36). Squamous cell carcinoma of the forestomach also occurred in two male mice exposed to 0.70 mM glycidamide. Squamous cell papillomas were characterized by a solitary stalk of lamina propria protruding into the forestomach lumen, with multiple finger-like projections arising from the stalk. The epithelium covering the projections usually displayed marked hyperplasia. Squamous cell carcinomas showed proliferation into the submucosa and in some cases had features of squamous cell differentiation, such as keratin production, while others were composed of large flattened cells typical of squamous morphology.

Male B6C3F1/Nctr mice exposed to glycidamide had a dose-related increase in squamous cell papilloma of the skin (Table 37), with the incidence being significant at 0.70 mM glycidamide. Squamous cell carcinoma of the skin also occurred in two male mice exposed to 0.70 mM glycidamide. The squamous cell papillomas arose from the epidermis as cauliflower-shaped or wart-like growths that featured a proliferating inner connective tissue core with an overlying proliferative squamous epithelium and an outer layer of keratin. The thickness of the epithelial layers varied from one papilloma to another. Mitotic figures were not uncommon. The squamous cell carcinomas appeared as ulcerated solid masses and arose from hyperplastic areas and borders of ulcers, with one arising from a papilloma. They were composed of irregular masses or cords of squamous epithelial cells that proliferated downward and invaded the adjacent dermis and subcutis. Keratin was present in these growths, with some forming characteristic epithelial pearls. The basal layers had frequent mitotic figures.

Female B6C3F1/Nctr mice had dose-related increases in malignant mesenchymal skin tumors (fibrosarcoma or combined fibrosarcoma or sarcoma; Table 37). The incidence of fibrosarcoma was significant at 0.70 mM glycidamide and the incidence of combined fibrosarcoma or sarcoma

was significant at 0.35 and 0.70 mM glycidamide. These mesenchymal tumors looked histologically similar to subcutaneous neoplasms previously seen in B6C3F1/Nctr mice.

Female B6C3F1/Nctr mice administered glycidamide in the drinking water had dose-related increasing trends in adenoacanthoma, adenocarcinoma, and combined adenoacanthoma or adenocarcinoma of the mammary gland (Table 38). The incidence of adenoacanthoma was increased significantly in the 0.70 mM glycidamide dose group and the incidence of adenocarcinoma and combined adenoacanthoma or adenocarcinoma was increased significantly in the 0.35 and 0.70 mM glycidamide dose groups. Mammary gland adenocarcinomas contained variably sized cystic structures lined by a pleomorphic to anaplastic cuboidal epithelium with frequent mitoses. Multiple growth patterns, such as acinar, tubular, solid, and papillary, were recognized. Adenoacanthomas showed similar features as carcinomas, except that at least 25% of the tumor consisted of squamous metaplasia. Female B6C3F1/Nctr mice also had dose-related increases in benign, malignant, and combined benign and malignant granulosa cell tumor of the ovary (Table 39).

Table 34. Incidences of Neoplasms and Nonneoplastic Lesions of the Eye in Mice in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Male					
Harderian Gland					
Adenoma, Bilateral	0/47	2/47	4/47	15/46	31/47
Adenoma (includes bilateral)[a]					
Overall rate[b]	3/47 (6%)	17/47 (36%)	23/47 (49%)	32/46 (70%)	42/47 (89%)
Poly-3 test[c]	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001
Eye					
Cataract					
Overall rate	1/47 (2%)	3/45 (7%)	7/46 (15%)	8/44 (18%)	17/42 (41%)
Poly-3 test	P < 0.001	P = 0.282	P = 0.019	P = 0.005	P < 0.001
Average severity[d]	4.0	1.0	2.1	2.3	2.0
Corneal Inflammation					
Overall rate	0/47 (0%)	0/45 (0%)	2/46 (4%)	0/44 (0%)	8/42 (19%)
Poly-3 test	P < 0.001	–	P = 0.209	–	P < 0.001
Average severity	–	–	2.5	–	2.1
Female					
Harderian Gland					
Adenoma, Bilateral	0/45	1/47	3/47	7/46	13/46
Adenoma (includes bilateral)[e]					
Overall rate	2/45 (4%)	19/47 (40%)	20/47 (43%)	24/46 (52%)	40/46 (87%)
Poly-3 test	P < 0.001	P < 0.001	P < 0.001	P < 0.001	P < 0.001

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Eye					
Cataract					
Overall rate	1/45 (2%)	2/44 (5%)	8/47 (17%)	8/44 (18%)	9/43 (21%)
Poly-3 test	P < 0.001	P = 0.496	P = 0.016	P = 0.010	P < 0.001
Average severity	1.0	3.0	3.1	2.5	1.6
Corneal Inflammation					
Overall rate	0/45 (0%)	2/44 (5%)	1/47 (2%)	3/44 (7%)	5/43 (12%)
Poly-3 test	P = 0.002	P = 0.238	P = 0.501	P = 0.103	P = 0.007
Average severity	–	2.0	1.0	1.3	2.2

[a]The historical incidence of Harderian gland adenoma in NCTR control male B6C3F1/Nctr mice is 5.9% (range 0.0–10.6%; Table C-3) for all studies and 5.7% (range 4.3–6.4%; Table C-3) for drinking water studies.
[b]Number of animals with neoplasm per number of animals examined microscopically.
[c]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice.
[d]Severity was scored as: 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.
[e]The historical incidence of Harderian gland adenoma in NCTR control female B6C3F1/Nctr mice is 5.0% (range 0.0–8.7%; Table D-5) for all studies and 4.4% (range 0.0–7.0%; Table D-5) for drinking water studies.

Table 35. Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Male					
Alveolar/Bronchiolar Adenoma, multiple					
	0/47	1/46	2/47	5/47	4/47
Alveolar/Bronchiolar Adenoma (including multiple)[a]					
Overall rate[b]	0/47 (0%)	7/46 (15%)	7/47 (15%)	13/47 (28%)	17/47 (36%)
Poly-3 test[c]	P < 0.001	P = 0.006	P = 0.005	P < 0.001	P < 0.001
Alveolar/Bronchiolar Carcinoma[d]					
Overall rate	0/47 (0%)	0/46 (0%)	1/47 (2%)	0/47 (0%)	2/47 (4%)
Poly-3 test	P = 0.058	–	P = 0.477	–	P = 0.196
Alveolar/Bronchiolar Adenoma or Carcinoma[e]					
Overall rate	0/47 (0%)	7/46 (15%)	8/47 (17%)	13/47 (28%)	19/47 (40%)
Poly-3 test	P < 0.001	P = 0.006	P = 0.002	P < 0.001	P < 0.001
Alveolar Epithelial Hyperplasia					
Overall rate	0/47	1/46	4/47	3/47	6/47
Poly-3 test	P = 0.003	P = 0.487	P = 0.048	P = 0.088	P = 0.008
Average severity[f]	–	2.0	2.3	3.3	2.5

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Female					
Alveolar/Bronchiolar Adenoma, multiple					
	0/46	0/48	0/47	0/47	2/44
Alveolar/Bronchiolar Adenoma (including multiple)[g]					
Overall rate	3/46 (7%)	5/48 (10%)	3/47 (6%)	7/47 (15%)	9/44 (21%)
Poly-3 test	P = 0.002	P = 0.363	P = 0.650	P = 0.139	P = 0.007
Alveolar/Bronchiolar Carcinoma[h]					
Overall rate	1/46 (2%)	1/48 (2%)	0/47 (0%)	1/47 (2%)	2/44 (5%)
Poly-3 test	P = 0.166	P = 0.755N	P = 0.505N	P = 0.744	P = 0.328
Alveolar/Bronchiolar Adenoma or Carcinoma[i]					
Overall rate	4/46 (9%)	6/48 (13%)	3/47 (6%)	8/47 (17%)	11/44 (25%)
Poly-3 test	P < 0.001	P = 0.385	P = 0.514N	P = 0.155	P = 0.003

[a]The historical incidence of alveolar/bronchiolar adenoma of the lung in NCTR control male B6C3F1/Nctr mice is 13.5% (range 6.3–27.1%; Table C-4) for all studies and 8.4% (range 6.3–10.6%; Table C-4) for drinking water studies.
[b]Number of animals with neoplasm per number of animals examined microscopically.
[c]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.
[d]The historical incidence of alveolar/bronchiolar carcinoma of the lung in NCTR control male B6C3F1/Nctr mice is 2.8% (range 0.0–8.3%; Table C-4) for all studies and 4.2% (range 2.1–6.3%; Table C-4) for drinking water studies.
[e]The historical incidence of alveolar/bronchiolar adenoma or carcinoma (combined) of the lung in NCTR control male B6C3F1/Nctr mice is 16.1% (range 10.4–31.3%; Table C-4) for all studies and 11.9% (range 10.4–12.8%; Table C-4) for drinking water studies.
[f]Severity was scored as: 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.
[g]The historical incidence of alveolar/bronchiolar adenoma of the lung in NCTR control female B6C3F1/Nctr mice is 4.7% (range 2.1–8.3%; Table D-3) for all studies and 5.7% (range 2.1–8.3%; Table D-3) for drinking water studies.
[h]The historical incidence of alveolar/bronchiolar carcinoma (combined) of the lung in NCTR control female B6C3F1/Nctr mice is 1.1% (range 0.0–4.3%; Table D-3) for all studies and 2.1% (range 0.0–4.2%; Table D-3) for drinking water studies.
[i]The historical incidence of alveolar/bronchiolar adenoma or carcinoma (combined) of the lung in NCTR control female B6C3F1/Nctr mice is 5.7% (range 2.1–12.5%; Table D-3) for all studies and 7.9% (range 4.3–12.5%; Table D-3) for drinking water studies.

Table 36. Incidences of Neoplasms and Nonneoplastic Lesions of the Stomach in Mice in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Male					
Forestomach, Squamous Cell Papilloma					
Overall rate[a]	0/47 (0%)	2/45 (4%)	3/48 (6%)	2/45 (4%)	10/41 (24%)
Poly-3 test[b]	P < 0.001	P = 0.218	P = 0.100	P = 0.193	P < 0.001
Forestomach, Squamous Cell Carcinoma					
Overall rate	0/47 (0%)	0/45 (0%)	0/48 (0%)	0/45 (0%)	2/41 (5%)
Poly-3 test	P = 0.014	–	–	–	0.176
Forestomach, Squamous Cell Papilloma or Carcinoma[c]					
Overall rate	0/47 (0%)	2/45 (4%)	3/48 (6%)	2/45 (4%)	12/41 (29%)
Poly-3 test	P < 0.001	P = 0.218	P = 0.100	P = 0.193	P < 0.001
Forestomach Epithelial Hyperplasia					
Overall rate	5/47	2/45	5/48	5/45	12/41
Poly-3 test	P < 0.001	P = 0.250N	P = 0.585	P = 0.493	P = 0.010
Average severity[d]	1.6	2.0	1.8	2.2	2.0
Female					
Forestomach, Squamous Cell Papilloma[e]					
Overall rate	1/45 (2%)	1/45 (2%)	1/47 (2%)	5/45 (11%)	9/44 (21%)
Poly-3 test	P < 0.001	P = 0.759	P = 0.759	P = 0.086	P < 0.001
Forestomach Epithelial Hyperplasia					
Overall rate	4/45 (9%)	4/45 (9%)	10/47 (21%)	11/45 (24%)	5/44 (11%)
Poly-3 test	P = 0.048	P = 0.641	P = 0.072	P = 0.028	P = 0.231
Average severity	3.0	2.8	2.4	2.5	2.4

[a]Number of animals with neoplasm per number of animals examined microscopically.
[b]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.
[c]The historical incidence of squamous cell papilloma or carcinoma (combined) of the forestomach in NCTR control male B6C3F1/Nctr mice is 0.3% (range 0.0–2.1%; Table C-5) for all studies and 0.0% (Table C-5) for drinking water studies.
[d]Severity was scored as: 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.
[e]The historical incidence of squamous cell papilloma or carcinoma (combined) of the forestomach in NCTR control female B6C3F1/Nctr mice is 1.6% (range 0.0–8.7%; Table D-7) for all studies and 4.4% (range 0.0–8.7%; Table D-7) for drinking water studies.

Table 37. Incidences of Neoplasms of the Skin in Mice in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Male					
Squamous Cell Papilloma					
Overall rate[a]	0/47 (0%)	1/48 (2%)	2/47 (4%)	1/47 (2%)	8/46 (17%)
Poly-3 test[b]	P < 0.001	P = 0.493	P = 0.213	P = 0.462	P = 0.001
Squamous Cell Carcinoma					
Overall rate	0/47 (0%)	0/48 (0%)	0/47 (0%)	0/47 (0%)	2/46 (4%)
Poly-3 test	P = 0.017	–	–	–	P = 0.191
Squamous Cell Papilloma or Carcinoma[c]					
Overall rate	0/47 (0%)	1/48 (2%)	2/47 (4%)	1/47 (2%)	9/46 (20%)
Poly-3 test	P < 0.001	P = 0.493	P = 0.213	P = 0.462	P < 0.001
Female					
Fibrosarcoma					
Overall rate	0/45 (0%)	1/48 (2%)	2/47 (4%)	2/47 (4%)	9/45 (20%)
Poly-3 test	P < 0.001	P = 0.506	P = 0.236	P = 0.229	P < 0.001
Sarcoma					
Overall rate	0/45 (0%)	0/48 (0%)	1/47 (2%)	3/47 (6%)	3/45 (7%)
Poly-3 test	P = 0.004	–	P = 0.499	P = 0.110	P = 0.059
Fibrosarcoma or Sarcoma[d]					
Overall rate	0/45 (0%)	1/48 (2%)	3/47 (6%)	5/47 (11%)	12/45 (27%)
Poly-3 test	P < 0.001	P = 0.506	P = 0.118	P = 0.028	P < 0.001

[a]Number of animals with neoplasm per number of animals examined microscopically.
[b]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice.
[c]The historical incidence of squamous cell papilloma or carcinoma (combined) of the skin in NCTR control male B6C3F1/Nctr mice is 0.2% (range 0.0–2.1%; Table C-7) for all studies and 0.7% (range 0.0–2.1%; Table C-7) for drinking water studies.
[d]The historical incidence of mescenchymal skin tumors in NCTR control female B6C3F1/Nctr mice is 1.7% (range 0.0–11.4%; Table D-6) for all studies and 6.4% (range 0.0–11.4%; Table D-6) for drinking water studies.

Table 38. Incidences of Neoplasms of the Mammary Gland in Female Mice in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Adenoacanthoma[a]					
Overall rate[b]	0/45 (0%)	0/48 (0%)	0/47 (0%)	1/47 (2%)	8/45 (18%)
Poly-3 test[c]	P < 0.001	–	–	P = 0.489	P < 0.001
Adenocarcinoma[d]					
Overall rate	1/45 (2%)	1/48 (2%)	2/47 (4%)	9/47 (19%)	11/45 (24%)
Poly-3 test	P < 0.001	P = 0.755N	P = 0.496	P = 0.006	P < 0.001

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Adenoacanthoma or Adenocarcinoma[e]					
Overall rate	1/45 (2%)	1/48 (2%)	2/47 (4%)	9/47 (19%)	18/45 (40%)
Poly-3 test	P < 0.001	P = 0.755N	P = 0.496	P = 0.006	P < 0.001

[a]The historical incidence of adenoacanthoma of the mammary gland in NCTR control female B6C3F1/Nctr mice is 0.4% (range 0.0–4.3%; Table D-4) for all studies and 0% (Table D-4) for drinking water studies.
[b]Number of animals with neoplasm per number of animals examined microscopically.
[c]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.
[d]The historical incidence of adenocarcinoma of the mammary gland in NCTR control female B6C3F1/Nctr mice is 3.3% (range 0.0–11.4%; Table D-4) for all studies and 6.5% (range 0.0–11.4%; Table D-4) for drinking water studies.
[e]The historical incidence of adenoacanthoma or adenocarcinoma of the mammary gland in NCTR control female B6C3F1/Nctr mice is 3.7% (range 0.0–11.4%; Table D-4) for all studies and 6.5% (range 0.0–11.4%; Table D-4) for drinking water studies.

Table 39. Incidences of Neoplasms and Nonneoplastic Lesions of the Reproductive System in Female Mice in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Ovary: Benign Granulosa Cell Tumor[a]					
Overall rate[b]	0/45 (0%)	0/47 (0%)	0/47 (0%)	1/46 (2%)	2/44 (5%)
Poly-3 test[c]	P = 0.014	–	–	P = 0.487	P = 0.146
Ovary: Malignant Granulosa Cell Tumor[d]					
Overall rate	0/45 (0%)	0/47 (0%)	0/47 (0%)	2/46 (4%)	1/44 (2%)
Poly-3 test	P = 0.048	–	–	P = 0.223	P = 0.402
Ovary: Benign or Malignant Granulosa Cell Tumor[e]					
Overall rate	0/45 (0%)	0/47 (0%)	0/47 (0%)	3/46 (4%)	3/44 (7%)
Poly-3 test	P = 0.001	–	–	P = 0.108	P = 0.055
Ovary: Cyst					
Overall rate	14/45	17/47	25/47	22/46	18/44
Poly-3 test	P = 0.011	P = 0.383	P = 0.016	P = 0.039	P = 0.022
Average severity[f]	2.6	2.6	2.9	2.9	2.9

[a]The historical incidence of benign granulosa cell tumor in NCTR control female B6C3F1/Nctr mice is 0.1% (range 0.0–0.7%; Table D-8) for all studies and 0% (Table D-8) for drinking water studies.
[b]Number of animals with neoplasm per number of animals examined microscopically.
[c]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice.
[d]The historical incidence of malignant granulosa cell tumor in NCTR control female B6C3F1/Nctr mice is 0.1% (range 0.0–0.6%; Table D-8) for all studies and 0% (Table D-8) for drinking water studies.
[e]The historical incidence of benign or malignant granulosa cell tumor in NCTR control female B6C3F1/Nctr mice is 0.3% (range 0.0–0.7%; Table D-8) for all studies and 0% (Table D-8) for drinking water studies.
[f]Severity was scored as: 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.

Nonneoplastic Findings

The drinking water administration of glycidamide to B6C3F1/Nctr mice resulted in cataracts in both sexes, with the incidence being significantly increased in the 0.175, 0.35, and 0.70 mM glycidamide dose groups (Table 34). Histopathologically, the cataracts displayed an irregular swelling of fiber cells with a granular vacuolated cytoplasm. Early mineralization was also noted along with disorganization of the lens epithelium. In addition, corneal inflammation was associated with glycidamide dosing in both male and female B6C3F1/Nctr mice, with the incidence being significant at 0.70 mM glycidamide.

Glycidamide administration resulted in a dose-related increasing trend in epithelial hyperplasia of the forestomach in both sexes of mice. In male mice, the incidence was significantly increased in the 0.70 mM glycidamide treatment group (Table 36). In female mice, the incidence was significantly increased in the 0.35 mM glycidamide dose group (Table 36). Focal squamous cell hyperplasia of the forestomach was evident as having multiple finger-like projections each with its own lamina propria and with excessive keratin on the surface. Focal rather than diffuse hyperplasia was the predominant pattern.

Both sexes of B6C3F1/Nctr mice had increasing dose-related trends in hematopoietic cell proliferation of the spleen, with the incidence being significantly increased at 0.35 and 0.70 mM glycidamide (Table 40 and Table 41). Hematopoietic cell proliferation was characterized in most animals by an increase in myeloid precursors, although erythroid hyperplasia was noted occasionally.

Other nonneoplastic lesions in male B6C3F1/Nctr mice included degeneration, ductal dilatation, and inflammation of the preputial gland (Table 40). Additional nonneoplastic lesions in female B6C3F1/Nctr mice included ovarian cysts (Table 39), hepatic angiectasis and necrosis, and axonal degeneration of the cervical spinal cord (Table 41). Hepatocellular necrosis had an irregular distribution of variably sized foci of hepatocytes that were either swollen with nuclear lysis or advanced featuring a condensed cytoplasm with minimal or no nuclear remnants present. A small inflammatory response was usually associated with this necrosis.

Table 40. Incidences of Selected Nonneoplastic Lesions in Male Mice in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Preputial Gland					
Degeneration					
Overall rate[a]	4/47 (9%)	10/47 (21%)	5/46 (11%)	12/46 (26%)	9/44 (21%)
Poly-3 test[b]	P = 0.041	P = 0.068	P = 0.419	P = 0.012	P = 0.048
Average severity[c]	2.8	2.9	3.8	3.3	3.1
Ductal Dilatation					
Overall rate	0/47 (0%)	0/47 (0%)	1/46 (2%)	0/46 (0%)	4/44 (9%)
Poly-3 test	P = 0.001	–	P = 0.473	–	P = 0.034
Average severity	–	–	4.0	–	3.5
Inflammation					
Overall rate	1/47 (2%)	6/47 (13%)	2/46 (4%)	3/46 (7%)	9/44 (21%)
Poly-3 test	P = 0.002	P = 0.047	P = 0.453	P = 0.237	P = 0.002
Average severity	1.0	2.2	2.5	2.0	2.8
Spleen					
Hematopoietic Cell Proliferation					
Overall rate	6/47 (13%)	6/47 (13%)	12/47 (26%)	14/46 (30%)	17/44 (39%)
Poly-3 test	P < 0.001	P = 0.587	P = 0.071	P = 0.016	P = 0.001
Average severity	3.7	3.2	3.3	3.1	3.1

[a]Number of animals with lesion per number of animals examined microscopically.
[b]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated (0.0875, 0.175, 0.35, and 0.70 mM glycidamide) group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice.
[c]Severity was graded as 1, minimal; 2, mild; 3, moderate; and 4, marked.

Table 41. Incidences of Selected Nonneoplastic Lesions in Female Mice in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Liver					
Angiectasis					
Overall rate[a]	0/47 (0%)	0/48 (0%)	1/47 (2%)	0/46 (0%)	5/43 (12%)
Poly-3 test[b]	P < 0.001	–	P = 0.491	–	P = 0.006
Average severity[c]	–	–	2.0	–	2.4
Necrosis					
Overall rate	0/47 (0%)	0/48 (0%)	0/47 (0%)	0/46 (0%)	5/43 (12%)
Poly-3 test	P < 0.001	–	–	–	P = 0.006
Average severity	–	–	–	–	2.2
Spinal Cord (Cervical)					
Axonal Degeneration					
Overall rate	4/45 (9%)	9/44 (21%)	10/47 (21%)	9/45 (20%)	10/43 (23%)
Poly-3 test	P = 0.011	P = 0.104	P = 0.069	P = 0.085	P = 0.005
Average severity	1.0	1.0	1.0	1.0	1.0
Spleen					
Hematopoietic Cell Proliferation					
Overall rate	6/46 (13%)	10/47 (21%)	11/47 (23%)	14/47 (30%)	29/45 (64%)
Poly-3 test	P < 0.001	P = 0.208	P = 0.144	P = 0.029	P < 0.001
Average severity	3.0	3.3	3.5	3.6	3.4

[a]Number of animals with lesion per number of animals examined microscopically.
[b]Beneath the 0 mM glycidamide are the p-values associated with the trend test. Beneath the treated (0.0875, 0.175, 0.35, and 0.70 mM glycidamide) group incidences are the p-values corresponding to pairwise comparisons between the 0 mM glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice.
[c]Severity was graded as 1, minimal; 2, mild; 3, moderate; and 4, marked.

Discussion

Glycidamide is a reactive electrophile that occurs primarily as a metabolite of acrylamide. Acrylamide can be formed by Maillard reactions involving asparagine and reducing sugars and thus is found as a contaminant in baked and fried starchy foods and roasted coffee. NTP conducted simultaneous studies to compare the effects of acrylamide and glycidamide in male and female F344/N Nctr rats and B6C3F1/Nctr mice. In NTP TR 575, acrylamide was shown to be a multi-site carcinogen in males and females of both species. Acrylamide was hypothesized to be activated to a carcinogen through its metabolism to glycidamide. The present study tests this hypothesis by comparing the effects of administering equimolar doses of glycidamide to the same rodent strains.

The administration of glycidamide in the drinking water to F344/N Nctr rats resulted in significant dose-related decreases in body weight, with the effect being most pronounced in the 0.70 mM glycidamide dose group (Table 10 and Table 11 and Figure 7). F344/N Nctr rats administered acrylamide in the drinking water for 2 years also had significant dose-related decreases in body weight[4], and a comparison of body weights between these two bioassays indicated that the mean body weights for all dose groups through the entire 2 year treatment period were typically within 5% of one another.

The survival of the F344/N Nctr rats also was affected by glycidamide, with significant decreases in survival being observed at the 0.35 and 0.70 mM doses (Table 9 and Figure 6). Similar trends existed with F344/N Nctr rats given acrylamide[4]. Furthermore, F344/N Nctr rats exposed to glycidamide or acrylamide typically consumed similar (±10%) amounts of each of the compounds (on a μMol per kg body weight basis; Table 42), thus permitting a direct comparison between the neoplasms arising as a result of the treatments.

Glycidamide induced follicular cell adenoma or carcinoma of the thyroid gland in both male and female F344/N Nctr rats. In male rats, the incidence of follicular cell adenoma or carcinoma was significantly increased at 0.70 mM glycidamide (Table 14) and in female rats, the incidence was increased significantly at 0.175, 0.35, and 0.70 mM glycidamide (Table 14); in both sexes the incidence in each of the glycidamide dose groups exceeded the historical range observed in control F344/N Nctr rats at the NCTR. Follicular cell carcinoma also was detected in each of the glycidamide dose groups of F344/N Nctr rats, except females administered 0.0875 mM glycidamide. Follicular cell carcinoma has not been observed in either male or female control F344/N Nctr rats in bioassays at the NCTR.

Follicular cell adenoma or carcinoma has been reported in F344 rats given acrylamide[4; 68; 69], and the incidence of follicular cell adenoma or carcinoma induced by acrylamide at NCTR[4] did not differ statistically from that induced by glycidamide (Table 43). In addition to having similar incidences of follicular cell neoplasms, F344 rats treated with equimolar levels of acrylamide or glycidamide have high levels of N7-GA-Gua in their thyroid gland DNA[21]. Furthermore, Big Blue rats administered equimolar quantities of acrylamide or glycidamide have increased mutant frequencies at the *cII* transgene of their thyroid glands[56]. Thus, the combined data are consistent with the conversion of acrylamide to glycidamide being an important step in the induction of follicular cell tumors in F344 rats.

The most sensitive site for tumor induction in female F344/N Nctr rats administered glycidamide in the drinking water was the mammary gland, where there was a significant increase in fibroadenoma at all dose levels of glycidamide (Table 19). Furthermore, the incidence of mammary gland fibroadenoma in each of the glycidamide dose groups exceeded the range observed in control female F344/N Nctr rats at the NCTR. The mammary gland was also the most sensitive site for tumor induction in female F344/N Nctr rats administered acrylamide in the drinking water[4] and the fibroadenoma incidences observed with glycidamide were very similar to those occurring with acrylamide (Table 43).

In addition to having similar incidences of mammary gland fibroadenomas, female F344 rats administered a single intraperitoneal injection of 0.7 mMole acrylamide or glycidamide per kg body weight form high levels of N7-GA-Gua in their mammary gland DNA[21], which supports the concept that the fibroadenomas result from a genotoxic mechanism as a consequence of the metabolic conversion of acrylamide to glycidamide. This interpretation conflicts with the fact that Big Blue rats treated with 0.12 mMol glycidamide or acrylamide per kg body weight/day for 2 months did not have an increased mutant frequency in the *cII* transgene in the mammary gland[56]; however, this may be a consequence of the high spontaneous mutant frequencies that occur with transgenic mutation assays in general.

Table 42. Comparison of Mean Daily Acrylamide and Glycidamide Consumption in Male and Female B6C3F1/Nctr Mice and F344/N Nctr Rats in Two-year Drinking Water Studies[a]

	Dose (mM)	Acrylamide (µmol/kg bw/day)	Glycidamide (µmol/kg bw/day)
F344/N Nctr Rats			
Male	0.0875***	4.6	4.4
	0.175*	9.1	8.9
	0.35***	18.4	17.6
	0.70	37.6	37.5
Female	0.0875	6.2	6.1
	0.175	12.3	12.3
	0.35*	25.8	25.4
	0.70***	56.1	52.6
B6C3F1/Nctr Mice			
Male	0.0875***	14.6	13.8
	0.175	30.9	30.4
	0.35	57.7	58.8
	0.70***	125.4	109.5
Female	0.0875	15.5	15.7
	0.175***	31.3	33.0
	0.35	65.3	64.6
	0.70**	139.7	147.6

[a]The data are reported as the mean amount consumed of acrylamide or glycidamide, in µMol kg body weight per day, for the entire 2-year experiment. An asterisk (*) associated with a specific dose indicates a significant (*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$) difference between acrylamide and glycidamide at the particular mM dose.

Table 43. Comparison of Incidences of Selected Neoplasms in Male and Female F344/N Nctr Rats Administered Acrylamide or Glycidamide in Two-year Drinking Water Studies[a]

	Dose (mM)	Acrylamide[b] (%)	Glycidamide (%)
Thyroid Gland: Follicular Cell Adenoma or Carcinoma			
Male	0	2	4
	0.0875	6	7
	0.175	9	13
	0.35	13	9
	0.70	19	28
Female	0	0	0
	0.0875	0	6
	0.175	4	11
	0.35	6	9
	0.70	9	17
Mammary Gland: Fibroadenoma			
Female	0**	33	33
	0.0875	38	54
	0.175*	52	73
	0.35**	47	69
	0.70	65	75
Clitoral Gland: Carcinoma			
Female	0*	2	8
	0.0875	13	13
	0.175	26	15
	0.35*	6	23
	0.70	17	30
Oral Mucosa or Tongue: Squamous Cell Papilloma or Carcinoma			
Female	0	0	2
	0.0875	4	4
	0.175	2	4
	0.35	6	4
	0.70	10	15
Epididymis or Testes: Malignant Mesothelioma			
Male	0**	4	0
	0.0875	4	2
	0.175	2	6
	0.35	10	21
	0.70*	17	35

	Dose (mM)	Acrylamide[b] (%)	Glycidamide (%)
Heart: Malignant Schwannoma			
Male	0	2	4
	0.0875	4	6
	0.175	6	6
	0.35	8	15
	0.70	13	17

[a]The data are reported the % incidence. An asterisk (*) associated with the 0 mM acrylamide or glycidamide indicates a significant (*, $p \leq 0.05$; **, $p \leq 0.01$) difference in regression line slopes. An asterisk (*) associated with a specific dose indicates a significant (*, $p \leq 0.05$; **, $p \leq 0.01$) difference between acrylamide and glycidamide at the particular mM dose.
[b]Data for acrylamide are from NTP TR 575[4].

Female F344/N Nctr rats treated with glycidamide in the drinking water also had increased incidences of clitoral gland carcinoma (Table 20) and oral cavity neoplasms (primarily squamous cell papilloma; Table 15) that, in each of the glycidamide dose groups, exceeded the NCTR historical range for control female F344/N Nctr rats. These neoplasms were also observed in female F344/N Nctr rats administered acrylamide in the drinking water[4], with incidences very similar to those recorded in the glycidamide bioassay (Table 43). Oral cavity neoplasms (primarily squamous cell papilloma) also occurred in male F344/N Nctr rats given glycidamide (Table 15). The incidence in the 0.70 mM glycidamide group was significant and the incidence in all dose groups, including the control group, exceeded the historical control range for male F344/N Nctr rats at the NCTR. These neoplasms were also observed in male F344/N Nctr rats administered acrylamide, but the incidences were not significant[4].

Female F344/N Nctr rats treated with 0.70 mM glycidamide had a low, but statistically significant, occurrence of forestomach papilloma (Table 21), the incidence of which exceeded the NCTR historical control range. Mononuclear cell leukemia was also observed in all dose groups of male and female F344/N Nctr rats, with the incidence in the 0.70 mM glycidamide group (Table 16) exceeding the NCTR historical control range for female F344/N Nctr rats.

In addition to thyroid gland and oral cavity neoplasms and leukemia, malignant mesothelioma of the epididymis or testes was observed in male F344/N Nctr rats administered glycidamide (Table 17). The incidence of these neoplasms was significant at 0.35 and 0.70 mM glycidamide and exceeded the NCTR historical control range. Malignant mesothelioma of the epididymis or testes has been reported in F344 rats given acrylamide[4; 68; 69] and the incidence of these neoplasms was similar to that induced by acrylamide at NCTR[4] except at the 0.70 mM dose, where the incidence was greater with glycidamide (Table 43). The higher tumor incidence in rats treated with 0.70 mM glycidamide compared to 0.70 mM acrylamide may reflect the saturation of enzymatic oxidation that occurs at high doses of acrylamide in rats[26]. Higher levels of N7-GA-Gua have been detected in testicular DNA from rats administered glycidamide as compared to acrylamide[21], which again may be a consequence of metabolic saturation occurring with acrylamide. Nonetheless, the concordance in tumor incidence that occurred at the lower doses supports the concept that the testicular tumors are a result of acrylamide being converted to glycidamide. Although these data suggest a genotoxic mechanism for the induction of testicular tumors, Big Blue rats treated with either acrylamide or glycidamide did not show an increased

mutant frequency in the testes[56]. This may be due to the fact that the entire testicular tissue rather than just the target tunica vaginalis was assessed.

Male F344/N Nctr rats administered glycidamide developed malignant schwannoma of the heart (Table 18), with the incidence in all dose groups, including the control group, exceeding the historical control range at the NCTR. This neoplasm was also observed in male F344/N Nctr rats treated with acrylamide[4]. In contrast, the increased incidence of adenoma or carcinoma of the pancreatic islets that was observed in male F344/N Nctr rats treated with acrylamide[4] was not detected in the current bioassay with glycidamide.

Special attention was given to the brain and spinal cord during the histopathological examinations because in one previous bioassay with acrylamide[69] tumors of glial cell origin were detected in the brain and spinal cord of male and female F344 rats. These tumors were not observed in two subsequent bioassays with acrylamide in F344 rats[4; 68] nor were they observed in the current bioassay with glycidamide. A treatment-related nonneoplastic lesion was gliosis, which was detected in both male and female F344/N Nctr rats (Table 22 and Table 23). A nonneoplastic lesion reported in F344 rats during previous 2-year bioassays with acrylamide was peripheral nerve degeneration[4; 68; 69]. An increased prevalence of this lesion was not observed in F344/N Nctr rats in the current study, although it should be noted that a high incidence of the lesion was detected in all dose groups, including the controls. Peripheral nerve degeneration was also observed in male and female F344/N Nctr rats administered 3.52 mM glycidamide for 3 months (Table 5). Rats from this group also displayed hind limb paresis. Hind limb paresis was also evident in both sexes of rats administered 7.03 mM glycidamide for 2 weeks (Table 5). Peripheral nerve degeneration was not evident in these rats. Hind limb paresis was not observed in any of the dose groups in the 2-year study.

The administration of glycidamide in the drinking water to B6C3F1/Nctr mice resulted in only sporadic changes in body weight (Table 30, Table 31 and Figure 11). The doses selected for the glycidamide bioassay (0, 0.0875, 0.175, 0.35, and 0.70 mM in the drinking water) were identical to those used in the acrylamide bioassay, and there were only sporadic differences in body weights when comparisons were made between mice administered acrylamide and mice given glycidamide. The survival of the B6C3F1/Nctr mice was affected by glycidamide, with significant decreases in survival being observed at the three highest doses in male mice and the two highest doses in female mice (Table 29). Similar trends existed with B6C3F1/Nctr mice given acrylamide[4]. In addition to having comparable body weights and survival, B6C3F1/Nctr mice exposed to glycidamide or acrylamide typically consumed similar (±10%) amounts of each of the compounds (on a μMol per kg body weight basis; Table 42). These results indicate that any differences in the incidence of neoplasms between mice given glycidamide and those administered acrylamide would not be a consequence of differences in body weights, survival, or amount of test compound consumed.

The most sensitive site for tumor induction in the B6C3F1/Nctr mice administered glycidamide in the drinking water was the Harderian gland. In male B6C3F1/Nctr mice, the incidence of Harderian gland adenoma increased from 6% in the control group to 89% in mice receiving 0.70 mM glycidamide in the drinking water (Table 34), and in female B6C3F1/Nctr mice, the incidence of Harderian gland adenoma increased from 4% in the control group to 87% in mice administered 0.70 mM glycidamide (Table 34). Even at the lowest dose of glycidamide (0.0875 mM), the incidence of Harderian gland adenoma exceeded the range observed in control

male and female B6C3F1/Nctr mice in experiments conducted at the NCTR. As reported previously, the Harderian gland was also the most sensitive site for tumor induction in the B6C3F1/Nctr mice administered acrylamide in the drinking water[4] and a comparison of the bioassays (Table 44) indicates that the tumor incidences were similar. These data, plus the fact that other low-molecular-weight carcinogens that are thought to be metabolized to electrophilic epoxides also target the Harderian gland in B6C3F1 mice[70-72], strongly support the concept that the carcinogenic activity of acrylamide in the Harderian gland of B6C3F1/Nctr mice is due to its metabolism to glycidamide.

In both sexes of B6C3F1/Nctr mice, there were significant dose-dependent increases in alveolar/bronchiolar adenoma of the lung (Table 35), and the incidences in the 0.35 and 0.70 mM glycidamide dose groups exceeded the range observed in control B6C3F1/Nctr mice in experiments at the NCTR. As with Harderian gland adenoma, the incidence of alveolar/bronchiolar adenoma in B6C3F1/Nctr mice administered glycidamide (Table 44) did not differ significantly from the incidence in mice given acrylamide[4]. These results, coupled with the observation that glycidamide and acrylamide give very similar DNA adduct profiles in the lungs of B6C3F1/Nctr and other strains of mice[17; 21; 23] are consistent with the premise that the lung neoplasms induced in B6C3F1/Nctr mice are due to acrylamide being metabolized to glycidamide.

In addition to Harderian gland and lung adenoma, the drinking water exposure to glycidamide resulted in significant dose-related increases in forestomach and skin neoplasms in both sexes of B6C3F1/Nctr mice and mammary gland neoplasms in female B6C3F1/Nctr mice. Forestomach neoplasms were also observed in male B6C3F1/Nctr mice administered acrylamide in the drinking water[4], and the incidence of these tumors did not differ significantly between the two compounds (Table 44). Likewise, skin and mammary gland neoplasms occurred in female B6C3F1/Nctr mice treated with acrylamide, and as was the case with forestomach neoplasms in male mice, the incidence of these neoplasms did not differ significantly between female mice given glycidamide and those treated with acrylamide. Other low-molecular-weight epoxides (e.g., glycidol[73]) or alkenes that are thought to be metabolized to electrophilic epoxides (e.g., 1,3-butadiene, chloroprene, and urethane[70; 72]) also induce mammary gland neoplasms in female B6C3F1 mice. Glycidol, 1,3-butadiene, and chloroprene also induce mammary gland tumors in female F344 rats[72; 73], as is the case with glycidamide (this report) and acrylamide[4].

Male B6C3F1 mice treated neonatally with glycidamide developed a high incidence of combined hepatocellular adenoma or carcinoma[38]. In other studies, high levels of N7-GA-Gua and N3-GA-Ade have been detected in liver DNA from mice and rats treated as adults with glycidamide[6; 7; 17; 21; 22], which suggests that glycidamide had the potential to be hepatocarcinogenic in the current bioassays. Liver necrosis was observed in male F344/N Nctr rats (Table 22) and female B6C3F1/Nctr mice (Table 41) administered glycidamide; nonetheless, an increased incidence of hepatocellular tumors was not observed in either species. F344/N Nctr rats and B6C3F1/Nctr mice treated with acrylamide also did not have increased incidences of liver neoplasms[4].

At the initiation of this study we hypothesized that acrylamide is carcinogenic due to its metabolic conversion to glycidamide. To determine the validity of this hypothesis, benchmark dose modeling was conducted to estimate the doses of glycidamide that would give a 10% excess risk for specific neoplasms (BMD); these doses were then compared to BMD values previously determined for acrylamide[74]. In F344/N Nctr rats, the most sensitive site for tumor induction was

the mammary gland in females (BMD of 2.39 µmol glycidamide per kg body weight per day for fibroadenoma) and the epididymis or testes in males (BMD of 11.23 to 17.94 µmol glycidamide per kg body weight per day for malignant mesothelioma) (Table K-1). In B6C3F1/Nctr mice, the most sensitive site for tumor induction by glycidamide was the Harderian gland, with a BMD for Harderian gland adenoma of 5.24 to 5.91 µmol glycidamide per kg body weight per day in males and 4.55 µmol glycidamide per kg body weight per day in females (Table K-2).

A comparison of these data with those previously reported for acrylamide[74] indicate that both chemicals have similar potencies in the target tissues (Table K-3 and Table K-4). For example, in female F344/N Nctr rats, the BMD for mammary gland fibroadenoma was 2.39 µMol per kg body weight per day for glycidamide and 7.74 µMol per kg body weight per day for acrylamide, and in male F344/N Nctr rats, the BMD for malignant mesothelioma of the epididymis or testes was 11.59 to 17.94 µMol per kg body weight per day for glycidamide and 29.90 to 30.66 µMol per kg body weight per day for acrylamide (Table K-3). The BMD for Harderian gland adenoma in male B6C3F1/Nctr mice was 5.51 to 5.91 µMol per kg body weight per day for glycidamide as compared to 5.14 to 5.39 µMol per kg body weight per day for acrylamide (Table K-4). Likewise, in female B6C3F1/Nctr mice the BMD for Harderian gland adenoma was 4.55 µMol per kg body weight per day for glycidamide as compared to 6.65 µMol per kg body weight per day for acrylamide. These data reinforce the concept that, under the conditions of these bioassays, acrylamide is efficiently metabolized to glycidamide in both sexes of both species and that the carcinogenic activity of acrylamide is due to its metabolic conversion into glycidamide.

We also hypothesized that because the metabolic conversion of acrylamide to glycidamide occurs to a greater extent in mice as compared to rats, mice would be more sensitive to the carcinogenic effects of acrylamide. The results from the benchmark dose modeling are in accord with this hypothesis. For instance, as noted previously the most sensitive site for tumor induction by acrylamide in B6C3F1/Nctr mice was the Harderian gland, with a BMD of 5.14 to 5.39 µmol acrylamide per kg body weight per day in males and 6.65 to 7.30 µmol acrylamide per kg body weight per day in females[74]. By comparison, the most sensitive site for tumor induction by acrylamide was the mammary gland in female F344/N Nctr rats (BMD of 7.74 to 12.86 µmol acrylamide per kg body weight per day) and the thyroid gland in male F344/N Nctr rats (BMD of 16.45 to 28.19 µmol acrylamide per kg body weight per day). Nonetheless, it is quite apparent that acrylamide and its electrophilic metabolite glycidamide induce a substantial neoplastic response in both species and sexes.

Table 44. Comparison of Incidences of Selected Neoplasms in Male and Female B6C3F1/Nctr Mice Administered Acrylamide or Glycidamide in Two-year Drinking Water Studies[a]

	Dose (mM)	Acrylamide[b] (%)	Glycidamide (%)
Harderian Gland: Adenoma			
Male	0	4	6
	0.0875	28	36
	0.175	57	49
	0.35	77	70
	0.70	83	89
Female	0	0	4
	0.0875*	18	40
	0.175	42	43
	0.35	68	52
	0.70	72	87
Lung: Alveolar/Bronchiolar Adenoma			
Male	0	11	0
	0.0875	13	15
	0.175	28	15
	0.35	22	28
	0.70	40	36
Female	0	2	7
	0.0875	9	10
	0.175	13	6
	0.35	24	15
	0.70	42	21
Stomach (Forestomach): Squamous Cell Papilloma or Carcinoma			
Male	0	0	0
	0.0875	4	4
	0.175	4	6
	0.35	15	4
	0.70	18	29
Skin: Mesenchymal Tumors			
Female	0	0	0
	0.0875	0	2
	0.175	6	6
	0.35	22	11
	0.70	14	27

	Dose (mM)	Acrylamide[b] (%)	Glycidamide (%)
Mammary Gland: Adenoacanthoma or Adenocarcinoma			
Female	0	0	2
	0.0875	9	2
	0.175	15	4
	0.35	9	19
	0.70	41	40

[a]The data are reported the % incidence. An asterisk (*) associated with a specific dose indicates a significant ($p \leq 0.05$) difference between acrylamide and glycidamide at the particular mM dose.
[b]Data for acrylamide are from NTP TR 575[4].

Conclusions

Under the conditions of this 2-year drinking water study, there was *clear evidence of carcinogenic activity*[a] of glycidamide in male F344/N Nctr rats based upon increased incidences of malignant mesothelioma of the epididymis and testis tunica, malignant schwannoma of the heart, follicular cell adenoma or carcinoma of the thyroid gland, and oral cavity (oral mucosa or tongue) squamous cell neoplasms (primarily papilloma). An increased incidence of mononuclear cell leukemia may have been related to acrylamide exposure. There was *clear evidence of carcinogenic activity* of glycidamide in female F344/N Nctr rats based upon increased incidences of mammary gland fibroadenoma, oral cavity (oral mucosa or tongue) squamous cell neoplasms (primarily papilloma), follicular cell adenoma or carcinoma of the thyroid gland, and carcinoma of the clitoral gland. Increased incidences of squamous cell papilloma of the forestomach and mononuclear cell leukemia were also considered to be related to glycidamide exposure.

There was *clear evidence of carcinogenic activity* of glycidamide in male B6C3F1/Nctr mice based upon increased incidences of adenoma of the Harderian gland, alveolar/bronchiolar adenoma of the lung, squamous cell neoplasms (primarily papilloma) of the skin and forestomach. There was *clear evidence of carcinogenic activity* of glycidamide in female B6C3F1/Nctr mice based upon increased incidences of adenoma of the Harderian gland, alveolar/bronchiolar adenoma of the lung, adenoacanthoma and adenocarcinoma of the mammary gland, squamous cell papilloma of the forestomach, and malignant mesenchymal neoplasms of the skin. The occurrence of granulosa cell tumor of the ovary may have been related to glycidamide exposure.

In F344/N Nctr rats, exposure to glycidamide was associated with increased incidence of brain gliosis (males and females), exfoliated germ cells within the epididymis (males), hepatocyte degeneration (males), liver necrosis (males), bone marrow hyperplasia (females), axonal degeneration of the lumbar spinal cord (females), and uterine endometrial hyperplasia (females).

In B6C3F1/Nctr mice, exposure to glycidamide was associated with increased incidences of cataracts (males and females), corneal inflammation (males and females), forestomach squamous cell hyperplasia (males and females), hematopoietic cell proliferation of the spleen (males and females), preputial gland lesions (degeneration, ductal dilatation, inflammation) (males), ovarian cysts (females), hepatic angiectasis and necrosis (females), and axonal degeneration of the cervical spinal cord (females).

The results of this bioassay, when compared to those previously reported for acrylamide, indicate that acrylamide is efficiently metabolized to glycidamide in both sexes of both species. Based upon the concordance of tumor sites between the two bioassays, the data also indicate that carcinogenic activity of acrylamide is due to its metabolic conversion to glycidamide.

[a]See Explanation of Levels of Evidence of Carcinogenic Activity. See summary of the peer review panel comments and the public discussion on this Technical Report in Appendix L.

References

1. Payne GB, Williams PH. Reactions of hydrogen peroxide. VI. Alkaline epoxidation of acrylonitrile. J Org Chem. 1961; 26(3):651-659. http://dx.doi.org/10.1021/jo01062a003

2. Becke F, Buckschewski H, Sander B, inventors. Production of glycidamide. United States patent 3217016; 1965

3. Shipp A, Lawrence G, Gentry R, McDonald T, Bartow H, Bounds J, Macdonald N, Clewell H, Allen B, Van Landingham C. Acrylamide: Review of toxicity data and dose-response analyses for cancer and noncancer effects. Crit Rev Toxicol. 2006; 36(6-7):481-608. http://dx.doi.org/10.1080/10408440600851377

4. National Toxicology Program (NTP). Toxicology and carcinogenesis studies of acrylamide (CASRN 79-06-1) in F344/N rats and B6C3F1 mice (feed and drinking water studies). Research Triangle Park, NC: National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services; 2012. Technical Report Series No. 575. NIH Publication No. 12-5917.

5. Granvogl M, Koehler P, Latzer L, Schieberle P. Development of a stable isotope dilution assay for the quantitation of glycidamide and its application to foods and model systems. J Agric Food Chem. 2008; 56(15):6087-6092. http://dx.doi.org/10.1021/jf800280b

6. Doerge DR, Young JF, McDaniel LP, Twaddle NC, Churchwell MI. Toxicokinetics of acrylamide and glycidamide in B6C3F1 mice. Toxicol Appl Pharmacol. 2005; 202(3):258-267. http://dx.doi.org/10.1016/j.taap.2004.07.001

7. Doerge DR, Young JF, McDaniel LP, Twaddle NC, Churchwell MI. Toxicokinetics of acrylamide and glycidamide in Fischer 344 rats. Toxicol Appl Pharmacol. 2005; 208(3):199-209. http://dx.doi.org/10.1016/j.taap.2005.03.003

8. Sumner SCJ, MacNeela JP, Fennell TR. Characterization and quantitation of urinary metabolites of [1,2,3-13C]acrylamide in rats and mice using carbon-13 nuclear magnetic resonance spectroscopy. Chem Res Toxicol. 1992; 5(1):81-89. http://dx.doi.org/10.1021/tx00025a014

9. Sumner SC, Fennell TR, Moore TA, Chanas B, Gonzalez F, Ghanayem BI. Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. Chem Res Toxicol. 1999; 12(11):1110-1116. http://dx.doi.org/10.1021/tx990040k

10. Doerge DR, Twaddle NC, Boettcher MI, McDaniel LP, Angerer J. Urinary excretion of acrylamide and metabolites in Fischer 344 rats and B6C3F(1) mice administered a single dose of acrylamide. Toxicol Lett. 2007; 169(1):34-42. http://dx.doi.org/10.1016/j.toxlet.2006.12.002

11. Fennell TR, Sumner SC, Snyder RW, Burgess J, Spicer R, Bridson WE, Friedman MA. Metabolism and hemoglobin adduct formation of acrylamide in humans. Toxicol Sci. 2005; 85(1):447-459. http://dx.doi.org/10.1093/toxsci/kfi069

12. Kopp EK, Dekant W. Toxicokinetics of acrylamide in rats and humans following single oral administration of low doses. Toxicol Appl Pharmacol. 2009; 235(2):135-142. http://dx.doi.org/10.1016/j.taap.2008.12.001

13. Sumner SC, Williams CC, Snyder RW, Krol WL, Asgharian B, Fennell TR. Acrylamide: A comparison of metabolism and hemoglobin adducts in rodents following dermal, intraperitoneal, oral, or inhalation exposure. Toxicol Sci. 2003; 75(2):260-270. http://dx.doi.org/10.1093/toxsci/kfg191

14. Kurebayashi H, Ohno Y. Metabolism of acrylamide to glycidamide and their cytotoxicity in isolated rat hepatocytes: Protective effects of GSH precursors. Arch Toxicol. 2006; 80(12):820-828. http://dx.doi.org/10.1007/s00204-006-0109-x

15. Backman J, Kronberg L. Reaction of glycidamide with 2'-deoxyadenosine and 2'-deoxyguanosine--mechanism for the amide hydrolysis. Nucleosides Nucleotides Nucleic Acids. 2007; 26(2):129-148. http://dx.doi.org/10.1080/15257770601112697

16. Backman J, Sjoholm R, Kronberg L. Characterization of the adducts formed in the reactions of glycidamide with thymidine and cytidine. Chem Res Toxicol. 2004; 17(12):1652-1658. http://dx.doi.org/10.1021/tx049823i

17. Gamboa da Costa G, Churchwell MI, Hamilton LP, Von Tungeln LS, Beland FA, Marques MM, Doerge DR. DNA adduct formation from acrylamide via conversion to glycidamide in adult and neonatal mice. Chem Res Toxicol. 2003; 16(10):1328-1337. http://dx.doi.org/10.1021/tx034108e

18. Kotova N, Juren T, Myohanen K, Cornelius M, Abramsson-Zetterberg L, Backman J, Menzel U, Rydberg P, Kronberg L, Vahakangas K et al. 32P-HPLC analysis of N1-(2-carboxy-2-hydroxyethyl)deoxyadenosine: A DNA adduct of the acrylamide-derived epoxide glycidamide. Toxicol Lett. 2011; 207(1):18-24. http://dx.doi.org/10.1016/j.toxlet.2011.08.007

19. Segerback D, Calleman CJ, Schroeder JL, Costa LG, Faustman EM. Formation of N-7-(2-carbamoyl-2-hydroxyethyl)guanine in DNA of the mouse and the rat following intraperitoneal administration of [14C]acrylamide. Carcinogenesis. 1995; 16(5):1161-1165. http://dx.doi.org/10.1093/carcin/16.5.1161

20. Solomon JJ. Cyclic adducts and intermediates induced by simple epoxides. In: Singer B, Bartsch, H, editors. Exocyclic DNA Adducts in Mutagenesis and Carcinogenesis. Lyon, France: IARC Sci Publ. 1999. p. 123-135.

21. Doerge DR, Gamboa da Costa G, McDaniel LP, Churchwell MI, Twaddle NC, Beland FA. DNA adducts derived from administration of acrylamide and glycidamide to mice and rats. Mutat Res Genet Toxicol Environ Mutagen. 2005; 580(1):131-141. http://dx.doi.org/10.1016/j.mrgentox.2004.10.013

22. Tareke E, Twaddle NC, McDaniel LP, Churchwell MI, Young JF, Doerge DR. Relationships between biomarkers of exposure and toxicokinetics in Fischer 344 rats and B6C3F1 mice administered single doses of acrylamide and glycidamide and multiple doses of acrylamide. Toxicol Appl Pharmacol. 2006; 217(1):63-75. http://dx.doi.org/10.1016/j.taap.2006.07.013

23. Von Tungeln LS, Churchwell MI, Doerge DR, Shaddock JG, McGarrity LJ, Heflich RH, Gamboa da Costa G, Marques MM, Beland FA. DNA adduct formation and induction of micronuclei and mutations in B6C3F1/Tk mice treated neonatally with acrylamide or glycidamide. Int J Cancer. 2009; 124(9):2006-2015. http://dx.doi.org/10.1002/ijc.24165

24. Martins C, Oliveira NG, Pingarilho M, Gamboa da Costa G, Martins V, Marques MM, Beland FA, Churchwell MI, Doerge DR, Rueff J et al. Cytogenetic damage induced by acrylamide and glycidamide in mammalian cells: Correlation with specific glycidamide-DNA adducts. Toxicol Sci. 2007; 95(2):383-390. http://dx.doi.org/10.1093/toxsci/kfl155

25. Mei N, Hu J, Churchwell MI, Guo L, Moore MM, Doerge DR, Chen T. Genotoxic effects of acrylamide and glycidamide in mouse lymphoma cells. Food Chem Toxicol. 2008; 46(2):628-636. http://dx.doi.org/10.1016/j.fct.2007.09.093

26. Bergmark E, Calleman CJ, Costa LG. Formation of hemoglobin adducts of acrylamide and its epoxide metabolite glycidamide in the rat. Toxicol Appl Pharmacol. 1991; 111(2):352-363. http://dx.doi.org/10.1016/0041-008X(91)90036-E

27. Calleman CJ, Bergmark E, Costa LG. Acrylamide is metabolized to glycidamide in the rat: Evidence from hemoglobin adduct formation. Chem Res Toxicol. 1990; 3(5):406-412. http://dx.doi.org/10.1021/tx00017a004

28. Bergmark E, Calleman CJ, He F, Costa LG. Determination of hemoglobin adducts in humans occupationally exposed to acrylamide. Toxicol Appl Pharmacol. 1993; 120(1):45-54. http://dx.doi.org/10.1006/taap.1993.1085

29. Annola K, Karttunen V, Keski-Rahkonen P, Myllynen P, Segerback D, Heinonen S, Vahakangas K. Transplacental transfer of acrylamide and glycidamide are comparable to that of antipyrine in perfused human placenta. Toxicol Lett. 2008; 182(1-3):50-56. http://dx.doi.org/10.1016/j.toxlet.2008.08.006

30. Annola K, Keski-Rahkonen P, Vähäkangas K, Lehtonen M. Simultaneous determination of acrylamide, its metabolite glycidamide and antipyrine in human placental perfusion fluid and placental tissue by liquid chromatography–electrospray tandem mass spectrometry. J Chromatogr B. 2008; 876(2):191-197. http://dx.doi.org/10.1016/j.jchromb.2008.10.044

31. Koyama N, Yasui M, Oda Y, Suzuki S, Satoh T, Suzuki T, Matsuda T, Masuda S, Kinae N, Honma M. Genotoxicity of acrylamide in vitro: Acrylamide is not metabolically activated in standard in vitro systems. Environ Mol Mutagen. 2011; 52(1):11-19. http://dx.doi.org/10.1002/em.20560

32. Costa LG, Deng H, Gregotti C, Manzo L, Faustman EM, Bergmark E, Calleman CJ. Comparative studies on the neuro- and reproductive toxicity of acrylamide and its epoxide metabolite glycidamide in the rat. Neurotoxicology. 1992; 13(1):219-224.

33. Abou-Donia MB, Ibrahim SM, Corcoran JJ, Lack L, Friedman MA, Lapadula DM. Neurotoxicity of glycidamide, an acrylamide metabolite, following intraperitoneal injections in rats. J Toxicol Environ Health. 1993; 39(4):447-464. http://dx.doi.org/10.1080/15287399309531764

34. Costa LG, Deng H, Calleman CJ, Bergmark E. Evaluation of the neurotoxicity of glycidamide, an epoxide metabolite of acrylamide: Behavioral, neurochemical and morphological studies. Toxicology. 1995; 98(1-3):151-161. http://dx.doi.org/10.1016/0300-483X(94)02986-5

35. Deng H, Jiao X, He F. [A study on neurotoxicity of acrylamide and glycidamide]. Chin J Prev Med. 1997; 31(4):202-205.

36. Brevik A, Rusnakova V, Duale N, Slagsvold HH, Olsen AK, Storeng R, Kubista M, Brunborg G, Lindeman B. Preconceptional paternal glycidamide exposure affects embryonic gene expression: Single embryo gene expression study following in vitro fertilization. Reprod Toxicol. 2011; 32(4):463-471. http://dx.doi.org/10.1016/j.reprotox.2011.09.005

37. Olstorn HB, Paulsen JE, Alexander J. Effects of perinatal exposure to acrylamide and glycidamide on intestinal tumorigenesis in Min/+ mice and their wild-type litter mates. Anticancer Res. 2007; 27(6b):3855-3864.

38. Von Tungeln LS, Doerge DR, Gamboa da Costa G, Matilde Marques M, Witt WM, Koturbash I, Pogribny IP, Beland FA. Tumorigenicity of acrylamide and its metabolite glycidamide in the neonatal mouse bioassay. Int J Cancer. 2012; 131(9):2008-2015. http://dx.doi.org/10.1002/ijc.27493

39. Hashimoto K, Tanii H. Mutagenicity of acrylamide and its analogues in Salmonella typhimurium. Mutat Res. 1985; 158(3):129-133. http://dx.doi.org/10.1016/0165-1218(85)90075-8

40. El-Assouli SM. Acrylamide in selected foods and genotoxicity of their extracts. J Egypt Public Health Assoc. 2009; 84(3-4):371-392.

41. Voogd CE, van der Stel JJ, Jacobs JJ. The mutagenic action of aliphatic epoxides. Mutat Res. 1981; 89(4):269-282. http://dx.doi.org/10.1016/0165-1218(81)90108-7

42. Barfknecht TR, Mecca DJ, Naismith RW. The genotoxic activity of acrylamide. Environ Mol Mutagen. 1988; 11 (Suppl 11):9.

43. Baum M, Fauth E, Fritzen S, Herrmann A, Mertes P, Merz K, Rudolphi M, Zankl H, Eisenbrand G. Acrylamide and glycidamide: Genotoxic effects in V79-cells and human blood. Mutat Res. 2005; 580(1-2):61-69. http://dx.doi.org/10.1016/j.mrgentox.2004.11.007

44. Baum M, Loeppky RN, Thielen S, Eisenbrand G. Genotoxicity of glycidamide in comparison to 3-N-nitroso-oxazolidin-2-one. J Agric Food Chem. 2008; 56(15):5989-5993. http://dx.doi.org/10.1021/jf703741a

45. Thielen S, Baum M, Hoffmann M, Loeppky RN, Eisenbrand G. Genotoxicity of glycidamide in comparison to (+/-)-anti-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide and alpha-acetoxy-N-nitroso-diethanolamine in human blood and in mammalian V79-cells. Mol Nutr Food Res. 2006; 50(4-5):430-436. http://dx.doi.org/10.1002/mnfr.200500227

46. Johansson F, Lundell T, Rydberg P, Erixon K, Jenssen D. Mutagenicity and DNA repair of glycidamide-induced adducts in mammalian cells. Mutat Res. 2005; 580(1-2):81-89. http://dx.doi.org/10.1016/j.mrgentox.2004.11.011

47. Besaratinia A, Pfeifer GP. Genotoxicity of acrylamide and glycidamide. J Natl Cancer Inst. 2004; 96(13):1023-1029. http://dx.doi.org/10.1093/jnci/djh186

48. Koyama N, Sakamoto H, Sakuraba M, Koizumi T, Takashima Y, Hayashi M, Matsufuji H, Yamagata K, Masuda S, Kinae N et al. Genotoxicity of acrylamide and glycidamide in human lymphoblastoid TK6 cells. Mutat Res. 2006; 603(2):151-158. http://dx.doi.org/10.1016/j.mrgentox.2005.11.006

49. Hansen SH, Olsen AK, Soderlund EJ, Brunborg G. In vitro investigations of glycidamide-induced DNA lesions in mouse male germ cells and in mouse and human lymphocytes. Mutat Res. 2010; 696(1):55-61. http://dx.doi.org/10.1016/j.mrgentox.2009.12.012

50. Puppel N, Tjaden Z, Fueller F, Marko D. DNA strand breaking capacity of acrylamide and glycidamide in mammalian cells. Mutat Res. 2005; 580(1-2):71-80. http://dx.doi.org/10.1016/j.mrgentox.2004.11.009

51. Butterworth BE, Eldridge SR, Sprankle CS, Working PK, Bentley KS, Hurtt ME. Tissue-specific genotoxic effects of acrylamide and acrylonitrile. Environ Mol Mutagen. 1992; 20(3):148-155. http://dx.doi.org/10.1002/em.2850200303

52. Generoso WM, Sega GA, Lockhart AM, Hughes LA, Cain KT, Cacheiro NL, Shelby MD. Dominant lethal mutations, heritable translocations, and unscheduled DNA synthesis induced in male mouse germ cells by glycidamide, a metabolite of acrylamide. Mutat Res. 1996; 371(3-4):175-183. http://dx.doi.org/10.1016/S0165-1218(96)90106-8

53. Paulsson B, Kotova N, Grawe J, Henderson A, Granath F, Golding B, Tornqvist M. Induction of micronuclei in mouse and rat by glycidamide, genotoxic metabolite of acrylamide. Mutat Res. 2003; 535(1):15-24. http://dx.doi.org/10.1016/S1383-5718(02)00281-4

54. Manjanatha MG, Aidoo A, Shelton SD, Bishop ME, McDaniel LP, Lyn-Cook LE, Doerge DR. Genotoxicity of acrylamide and its metabolite glycidamide administered in drinking water to male and female Big Blue mice. Environ Mol Mutagen. 2006; 47(1):6-17. http://dx.doi.org/10.1002/em.20157

55. Wang RS, McDaniel LP, Manjanatha MG, Shelton SD, Doerge DR, Mei N. Mutagenicity of acrylamide and glycidamide in the testes of Big Blue mice. Toxicol Sci. 2010; 117(1):72-80. http://dx.doi.org/10.1093/toxsci/kfq190

56. Mei N, McDaniel LP, Dobrovolsky VN, Guo X, Shaddock JG, Mittelstaedt RA, Azuma M, Shelton SD, McGarrity LJ, Doerge DR et al. The genotoxicity of acrylamide and glycidamide in Big Blue rats. Toxicol Sci. 2010; 115(2):412-421. http://dx.doi.org/10.1093/toxsci/kfq069

57. Twaddle NC, Churchwell MI, McDaniel LP, Doerge DR. Autoclave sterilization produces acrylamide in rodent diets: Implications for toxicity testing. J Agric Food Chem. 2004; 52(13):4344-4349. http://dx.doi.org/10.1021/jf0497657

58. Maronpot RR, Boorman GA. Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. Toxicol Pathol. 1982; 10(2):71-78. http://dx.doi.org/10.1177/019262338201000210

59. Boorman GA, Montgomery CA, Jr., Eustis SL, Wolfe MJ, McConnell EE, Hardisty JF. Quality assurance in pathology for rodent carcinogenicity studies In: Milman HA, Weisburger EK, editors. Handbook of Carcinogen Testing. Park Ridge, NJ: Noyes Publications; 1985. p. 345-357.

60. McConnell EE, Solleveld HA, Swenberg JA, Boorman GA. Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. J Natl Cancer Inst. 1986; 76(2):283-289.

61. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Amer Stat Assoc. 1958; 53(282):457-481. http://dx.doi.org/10.1080/01621459.1958.10501452

62. Cox DR. Regression models and life-tables. J Royal Stat Soc B. 1972; 34:187-220.

63. Dunnett CW. A multiple comparison procedure for comparing several treatments with a control. J Amer Stat Assoc. 1955; 50(272):1096-1121. http://dx.doi.org/10.1080/01621459.1955.10501294

64. Bailer AJ, Portier CJ. Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. Biometrics. 1988; 44(2):417-431. http://dx.doi.org/10.2307/2531856

65. Bieler GS, Williams RL. Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity. Biometrics. 1993; 49(3):793-801. http://dx.doi.org/10.2307/2532200

66. Haseman JK. Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. Environ Health Perspect. 1984; 58:385-392. http://dx.doi.org/10.1289/ehp.8458385

67. Code of Federal Regulations (CFR). 21:Part 58.

68. Friedman MA, Dulak LH, Stedham MA. A lifetime oncogenicity study in rats with acrylamide. Fundam Appl Toxicol. 1995; 27(1):95-105. http://dx.doi.org/10.1006/faat.1995.1112

69. Johnson KA, Gorzinski SJ, Bodner KM, Campbell RA, Wolf CH, Friedman MA, Mast RW. Chronic toxicity and oncogenicity study on acrylamide incorporated in the drinking water of Fischer 344 rats. Toxicol Appl Pharmacol. 1986; 85(2):154-168. http://dx.doi.org/10.1016/0041-008X(86)90109-2

70. Beland FA, Benson RW, Mellick PW, Kovatch RM, Roberts DW, Fang JL, Doerge DR. Effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in B6C3F1 mice. Food Chem Toxicol. 2005; 43(1):1-19. http://dx.doi.org/10.1016/j.fct.2004.07.018

71. Bucher JR, Huff J, Haseman JK, Eustis SL, Peters A, Toft JD. Neurotoxicity and carcinogenicity of N-methylolacrylamide in F344 rats and B6C3F1 mice. J Toxicol Environ Health. 1990; 31(3):161-177. http://dx.doi.org/10.1080/15287399009531446

72. Melnick RL, Sills RC. Comparative carcinogenicity of 1,3-butadiene, isoprene, and chloroprene in rats and mice. Chem Biol Interact. 2001; 135-136:27-42. http://dx.doi.org/10.1016/S0009-2797(01)00213-7

73. Irwin RD, Eustis SL, Stefanski S, Haseman JK. Carcinogenicity of glycidol in F344 rats and B6C3F1 mice. J Appl Toxicol. 1996; 16(3):201-209. http://dx.doi.org/10.1002/(SICI)1099-1263(199605)16:3<201::AID-JAT333>3.0.CO;2-0

74. Beland FA, Mellick PW, Olson GR, Mendoza MC, Marques MM, Doerge DR. Carcinogenicity of acrylamide in B6C3F$_1$ mice and F344/N rats from a 2-year drinking water exposure. Food Chem Toxicol. 2013; 51:149-159. http://dx.doi.org/10.1016/j.fct.2012.09.017

75. Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environ Mol Mutagen. 1992; 19 Suppl 21:2-141. http://dx.doi.org/10.1002/em.2850190603

76. European Food Safety Authority (EFSA). Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. EFSA Journal. 2009; 1150:1-72. http://dx.doi.org/10.2903/j.efsa.2009.1150

Appendix A. Summary of Lesions in Male Rats in the Two-year Drinking Water Study of Glycidamide

Tables

Table A-1. Summary of the Incidence of Neoplasms in Male Rats in the Two-year Drinking Water Study of Glycidamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Disposition Summary					
Animals initially in study	48	48	48	48	48
Early deaths					
Moribund sacrifice	16	13	24	26	36
Natural deaths	4	10	4	2	1
Survivors					
Moribund sacrifice	7	5	4	10	7
Natural deaths	–	2	1	2	2
Terminal sacrifice	21	18	15	7	2
Other	–	–	–	1	–
Animals examined microscopically	48	48	48	47	48
Alimentary System					
Esophagus	(48)	(47)	(48)	(47)	(48)
Intestine large, cecum	(45)	(37)	(43)	(42)	(46)
Leukemia mononuclear	1 (2%)	–	–	2 (5%)	–
Intestine large, colon	(46)	(38)	(44)	(43)	(45)
Adenocarcinoma	–	1 (3%)	–	–	–
Leukemia mononuclear	–	–	–	1 (2%)	1 (2%)
Lymphoma malignant	–	–	1 (2%)	–	–
Intestine large, rectum	(46)	(37)	(44)	(44)	(45)
Intestine small, duodenum	(46)	(37)	(43)	(43)	(46)
Leukemia mononuclear	–	–	–	1 (2%)	1 (2%)
Lymphoma malignant	–	–	1 (2%)	–	–
Intestine small, ileum	(45)	(37)	(43)	(43)	(45)
Histiocytic sarcoma	–	–	–	1 (2%)	–
Leukemia mononuclear	–	–	–	1 (2%)	1 (2%)
Lymphoma malignant	–	–	1 (2%)	–	–
Serosa, schwannoma malignant	–	–	1 (2%)	–	–
Intestine small, jejunum	(45)	(36)	(43)	(43)	(45)
Adenocarcinoma	–	–	–	1 (2%)	–
Leiomyoma	–	–	–	1 (2%)	–
Liver	(47)	(47)	(48)	(47)	(47)
Hepatocellular adenoma	1 (2%)	–	–	–	2 (4%)
Hepatocellular adenoma, multiple	–	1 (2%)	–	–	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Histiocytic sarcoma	–	–	–	1 (2%)	1 (2%)
Leukemia mononuclear	20 (43%)	24 (51%)	26 (54%)	27 (57%)	30 (64%)
Lymphoma malignant	–	–	1 (2%)	–	–
Mesentery	(3)	(8)	(6)	(9)	(4)
Adenocarcinoma, metastatic, intestine small, jejunum	–	–	–	1 (11%)	–
Hemangioma	–	1 (13%)	–	–	–
Leukemia mononuclear	1 (33%)	4 (50%)	–	3 (33%)	1 (25%)
Lipoma	1 (33%)	–	–	–	–
Mesothelioma malignant	–	–	–	1 (11%)	2 (50%)
Oral mucosa	(4)	(3)	(2)	(6)	(7)
Squamous cell carcinoma	1 (25%)	–	1 (50%)	1 (17%)	–
Squamous cell papilloma	1 (25%)	1 (33%)	–	2 (33%)	3 (43%)
Pancreas	(48)	(44)	(47)	(46)	(48)
Leukemia mononuclear	1 (2%)	5 (11%)	2 (4%)	5 (11%)	5 (10%)
Lymphoma malignant	–	–	1 (2%)	–	–
Mesothelioma malignant	–	–	–	1 (2%)	3 (6%)
Sarcoma	1 (2%)	–	–	–	–
Acinar cell, adenoma	–	1 (2%)	1 (2%)	–	–
Salivary glands	(48)	(44)	(48)	(46)	(47)
Leukemia mononuclear	2 (4%)	1 (2%)	–	2 (4%)	–
Lymphoma malignant	–	–	1 (2%)	–	–
Schwannoma malignant	–	–	–	–	1 (2%)
Stomach, forestomach	(46)	(46)	(46)	(47)	(47)
Leukemia mononuclear	–	–	–	2 (4%)	–
Lymphoma malignant	–	–	1 (2%)	–	–
Stomach, glandular	(46)	(38)	(44)	(44)	(46)
Leukemia mononuclear	1 (2%)	1 (3%)	–	2 (5%)	2 (4%)
Lymphoma malignant	–	–	1 (2%)	–	–
Tongue	(0)	(1)	(1)	(2)	(6)
Squamous cell carcinoma	–	–	1 (100%)	–	–
Squamous cell papilloma	–	1 (100%)	–	1 (50%)	4 (67%)
Cardiovascular System					
Blood vessel	(48)	(48)	(48)	(47)	(48)
Leukemia mononuclear	–	–	1 (2%)	5 (11%)	3 (6%)
Lymphoma malignant	–	–	1 (2%)	–	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Mesothelioma malignant	–	–	1 (2%)	–	–
Heart	(48)	(48)	(48)	(47)	(48)
Histiocytic sarcoma	–	–	–	–	1 (2%)
Leukemia mononuclear	12 (25%)	18 (38%)	14 (29%)	16 (34%)	24 (50%)
Lymphoma malignant	–	–	1 (2%)	–	–
Mesothelioma malignant	–	–	1 (2%)	–	–
Schwannoma malignant	2 (4%)	3 (6%)	3 (6%)	7 (15%)	8 (17%)
Endocrine System					
Adrenal cortex	(47)	(47)	(48)	(47)	(47)
Histiocytic sarcoma	–	–	–	–	1 (2%)
Leukemia mononuclear	3 (6%)	5 (11%)	–	2 (4%)	8 (17%)
Lymphoma malignant	–	–	1 (2%)	–	–
Mesothelioma malignant	–	–	–	–	1 (2%)
Adrenal medulla	(46)	(47)	(48)	(47)	(48)
Leukemia mononuclear	4 (9%)	9 (19%)	6 (13%)	11 (23%)	8 (17%)
Pheochromocytoma benign	8 (17%)	10 (21%)	13 (27%)	3 (6%)	7 (15%)
Pheochromocytoma complex	2 (4%)	1 (2%)	–	–	–
Pheochromocytoma malignant	4 (9%)	1 (2%)	4 (8%)	–	2 (4%)
Bilateral, pheochromocytoma benign	2 (4%)	1 (2%)	1 (2%)	5 (11%)	2 (4%)
Islets, pancreatic	(48)	(45)	(48)	(45)	(48)
Adenoma	4 (8%)	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Carcinoma	1 (2%)	–	–	–	–
Leukemia mononuclear	–	–	–	2 (4%)	1 (2%)
Lymphoma malignant	–	–	1 (2%)	–	–
Parathyroid gland	(48)	(47)	(47)	(45)	(48)
Pituitary gland	(47)	(46)	(48)	(47)	(47)
Leukemia mononuclear	5 (11%)	9 (20%)	4 (8%)	6 (13%)	3 (6%)
Pars distalis, adenoma	28 (60%)	22 (48%)	31 (65%)	21 (45%)	19 (40%)
Pars distalis, adenoma, multiple	–	1 (2%)	–	–	1 (2%)
Pars distalis, carcinoma	–	–	–	1 (2%)	–
Pars intermedia, adenoma	1 (2%)	1 (2%)	–	–	–
Thyroid gland	(47)	(42)	(48)	(47)	(46)
Leukemia mononuclear	1 (2%)	–	–	–	–
Lymphoma malignant	–	–	1 (2%)	–	–
C-cell, adenoma	4 (9%)	–	1 (2%)	–	1 (2%)
C-cell, carcinoma	1 (2%)	2 (5%)	1 (2%)	1 (2%)	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Follicular cell, adenoma	2 (4%)	1 (2%)	3 (6%)	3 (6%)	7 (15%)
Follicular cell, adenoma, multiple	–	–	–	–	1 (2%)
Follicular cell, carcinoma	–	2 (5%)	3 (6%)	1 (2%)	5 (11%)
General Body System					
Tissue NOS	(2)	(0)	(1)	(1)	(1)
Histiocytic sarcoma	–	–	–	1 (100%)	–
Leukemia mononuclear	1 (50%)	–	–	–	–
Schwannoma malignant	–	–	1 (100%)	–	–
Mediastinum, leukemia mononuclear	–	–	–	–	1 (100%)
Thoracic, sarcoma	1 (50%)	–	–	–	–
Genital System					
Coagulating gland	(0)	(0)	(0)	(0)	(1)
Epididymis	(48)	(45)	(48)	(47)	(47)
Leukemia mononuclear	–	–	–	1 (2%)	–
Lymphoma malignant	–	–	1 (2%)	–	–
Mesothelioma malignant	–	1 (2%)	3 (6%)	10 (21%)	17 (36%)
Adventitia, schwannoma malignant	–	–	1 (2%)	–	–
Preputial gland	(48)	(48)	(48)	(47)	(48)
Adenoma	2 (4%)		5 (10%)	3 (6%)	2 (4%)
Carcinoma	6 (13%)	1 (2%)	2 (4%)	6 (13%)	4 (8%)
Hemangioma	–	–	–	1 (2%)	–
Leukemia mononuclear	3 (6%)	–	1 (2%)	2 (4%)	1 (2%)
Schwannoma malignant	–	–	–	–	1 (2%)
Squamous cell carcinoma	6 (13%)	5 (10%)	4 (8%)	4 (9%)	3 (6%)
Squamous cell papilloma	–	1 (2%)	–	–	–
Prostate	(47)	(47)	(48)	(47)	(48)
Adenoma	1 (2%)	1 (2%)	–	3 (6%)	1 (2%)
Histiocytic sarcoma	–	–	–	1 (2%)	–
Leukemia mononuclear	2 (4%)	2 (4%)	–	2 (4%)	–
Lymphoma malignant	–	–	1 (2%)	–	–
Mesothelioma malignant	–	–	–	1 (2%)	1 (2%)
Seminal vesicle	(47)	(38)	(46)	(46)	(47)
Leukemia mononuclear	–	–	–	1 (2%)	–
Lymphoma malignant	–	–	1 (2%)	–	–
Mesothelioma malignant	–	–	–	1 (2%)	2 (4%)
Adventitia, sarcoma	1 (2%)	–	–	–	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Testes	(48)	(47)	(48)	(47)	(48)
Leukemia mononuclear	–	–	–	1 (2%)	–
Mesothelioma malignant	–	1 (2%)	3 (6%)	6 (13%)	13 (27%)
Bilateral, interstitial cell, adenoma	22 (46%)	17 (36%)	17 (35%)	21 (45%)	10 (21%)
Interstitial cell, adenoma	11 (23%)	17 (36%)	16 (33%)	13 (28%)	17 (35%)
Hematopoietic System					
Bone marrow	(47)	(40)	(45)	(46)	(46)
Histiocytic sarcoma	–	–	–	1 (2%)	1 (2%)
Leukemia mononuclear	14 (30%)	18 (45%)	16 (36%)	17 (37%)	23 (50%)
Lymph node	(17)	(19)	(19)	(23)	(23)
Leukemia mononuclear	1 (6%)	1 (5%)	–	–	–
Axillary, leukemia mononuclear	3 (18%)	2 (11%)	1 (5%)	5 (22%)	1 (4%)
Brachial, leukemia mononuclear	–	1 (5%)	–	–	–
Cervical, leukemia mononuclear	–	–	–	1 (4%)	–
Lumbar, leukemia mononuclear	5 (29%)	7 (37%)	5 (26%)	5 (22%)	4 (17%)
Lumbar, squamous cell carcinoma, metastatic, preputial gland	1 (6%)	–	–	–	–
Mediastinal, histiocytic sarcoma	–	–	–	1 (4%)	–
Mediastinal, leukemia mononuclear	7 (41%)	8 (42%)	4 (21%)	7 (30%)	7 (30%)
Mediastinal, lymphoma malignant	–	–	1 (5%)	–	–
Pancreatic, histiocytic sarcoma	–	–	–	1 (4%)	–
Pancreatic, leukemia mononuclear	7 (41%)	10 (53%)	5 (26%)	9 (39%)	11 (48%)
Pancreatic, lymphoma malignant	–	–	1 (5%)	–	–
Renal, histiocytic sarcoma	–	–	–	1 (4%)	–
Renal, leukemia mononuclear	6 (35%)	5 (26%)	5 (26%)	8 (35%)	4 (17%)
Thoracic, leukemia mononuclear	–	–	–	–	1 (4%)
Lymph node, mandibular	(48)	(45)	(48)	(47)	(47)
Histiocytic sarcoma	–	–	–	1 (2%)	–
Leukemia mononuclear	14 (29%)	17 (38%)	17 (35%)	16 (34%)	21 (45%)
Lymphoma malignant	–	–	1 (2%)	–	–
Follicular, carcinoma, metastatic, thyroid gland	–	1 (2%)	–	–	–
Lymph node, mesenteric	(48)	(46)	(46)	(46)	(47)
Histiocytic sarcoma	–	–	–	1 (2%)	1 (2%)
Leukemia mononuclear	16 (33%)	19 (41%)	20 (43%)	20 (43%)	22 (47%)
Lymphoma malignant	–	–	1 (2%)	–	–
Spleen	(48)	(47)	(48)	(47)	(47)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Histiocytic sarcoma	–	–	–	1 (2%)	1 (2%)
Leukemia mononuclear	21 (44%)	25 (53%)	26 (54%)	27 (57%)	31 (66%)
Mesothelioma malignant	–	–	–	–	2 (4%)
Thymus	(43)	(38)	(43)	(42)	(43)
Histiocytic sarcoma	–	–	–	1 (2%)	–
Leukemia mononuclear	3 (7%)	3 (8%)	3 (7%)	7 (17%)	4 (9%)
Lymphoma malignant	–	–	1 (2%)	–	–
Integumentary System					
Mammary gland	(45)	(42)	(45)	(44)	(42)
Adenocarcinoma	2 (4%)	–	–	1 (2%)	–
Adenoma	–	–	1 (2%)	–	–
Fibroadenoma	4 (9%)	6 (14%)	3 (7%)	5 (11%)	6 (14%)
Fibroadenoma, multiple	–	–	–	2 (5%)	–
Leukemia mononuclear	1 (2%)	–	–	1 (2%)	–
Skin	(48)	(48)	(48)	(47)	(48)
Basal cell adenoma	1 (2%)	–	2 (4%)	1 (2%)	–
Keratoacanthoma	1 (2%)	1 (2%)	–	1 (2%)	2 (4%)
Leukemia mononuclear	1 (2%)	–	–	1 (2%)	–
Squamous cell papilloma	–	–	–	–	2 (4%)
Ear, squamous cell papilloma	–	–	–	1 (2%)	–
Sebaceous gland, adenoma	–	1 (2%)	–	–	–
Subcutaneous tissue, fibroma	2 (4%)	–	1 (2%)	1 (2%)	4 (8%)
Subcutaneous tissue, lipoma	–	–	1 (2%)	1 (2%)	–
Subcutaneous tissue, schwannoma malignant	–	–	1 (2%)	1 (2%)	–
Musculoskeletal System					
Bone	(1)	(0)	(2)	(3)	(1)
Cranium, osteoma	–	–	–	1 (33%)	1 (100%)
Humerus, osteosarcoma	–	–	1 (50%)	–	–
Vertebra, leukemia mononuclear	–	–	–	1 (33%)	–
Bone, femur	(48)	(48)	(48)	(47)	(48)
Skeletal muscle	(48)	(48)	(48)	(47)	(48)
Mesothelioma malignant	–	–	–	–	2 (4%)
Nervous System					
Brain, brain stem	(48)	(48)	(48)	(47)	(48)
Histiocytic sarcoma	1 (2%)	–	–	–	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Leukemia mononuclear	1 (2%)	8 (17%)	3 (6%)	4 (9%)	1 (2%)
Brain, cerebellum	(48)	(48)	(48)	(47)	(48)
Histiocytic sarcoma	1 (2%)	–	–	–	–
Leukemia mononuclear	2 (4%)	7 (15%)	5 (10%)	5 (11%)	2 (4%)
Brain, cerebrum	(48)	(48)	(48)	(47)	(48)
Glioma malignant	1 (2%)	–	–	–	–
Histiocytic sarcoma	1 (2%)	–	–	–	–
Leukemia mononuclear	2 (4%)	7 (15%)	5 (10%)	4 (9%)	–
Meninges, lymphoma malignant	–	–	1 (2%)	–	–
Peripheral nerve, sciatic	(48)	(48)	(48)	(47)	(48)
Spinal cord, cervical	(47)	(46)	(48)	(47)	(48)
Leukemia mononuclear	2 (4%)	2 (4%)	5 (10%)	4 (9%)	–
Spinal cord, lumbar	(47)	(46)	(48)	(47)	(48)
Leukemia mononuclear	2 (4%)	2 (4%)	5 (10%)	5 (11%)	–
Spinal cord, thoracic	(47)	(46)	(48)	(47)	(48)
Leukemia mononuclear	–	1 (2%)	3 (6%)	1 (2%)	–
Respiratory System					
Lung	(48)	(47)	(48)	(47)	(47)
Alveolar/bronchiolar adenoma	–	1 (2%)	–	–	1 (2%)
Carcinoma, metastatic, thyroid gland	–	–	–	1 (2%)	–
Carcinoma, metastatic, Zymbal's gland	–	–	–	1 (2%)	–
Histiocytic sarcoma	–	–	–	1 (2%)	1 (2%)
Leukemia mononuclear	13 (27%)	21 (45%)	17 (35%)	19 (40%)	22 (47%)
Lymphoma malignant	–	–	1 (2%)	–	–
Mesothelioma malignant	–	–	1 (2%)	–	–
Osteosarcoma, metastatic, bone	–	–	1 (2%)	–	–
Pheochromocytoma malignant, metastatic, adrenal medulla	1 (2%)	–	–	–	–
Alveolar epithelium, alveolar/bronchiolar adenoma	–	–	–	–	1 (2%)
Nose	(48)	(45)	(47)	(45)	(48)
Osteosarcoma	–	–	–	1 (2%)	–
Mucosa, squamous cell carcinoma	–	–	1 (2%)	–	–
Trachea	(48)	(43)	(48)	(47)	(47)
Special Senses System					
Ear	(0)	(0)	(1)	(1)	(0)
Neural crest tumor	–	–	1 (100%)	1 (100%)	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Eye	(45)	(38)	(43)	(44)	(45)
Leukemia mononuclear	–	–	–	1 (2%)	–
Melanoma malignant	–	–	–	1 (2%)	–
Harderian gland	(47)	(47)	(48)	(46)	(48)
Leukemia mononuclear	1 (2%)	–	1 (2%)	1 (2%)	–
Zymbal's gland	(0)	(0)	(3)	(2)	(3)
Carcinoma	–	–	3 (100%)	2 (100%)	3 (100%)
Urinary System					
Kidney	(46)	(44)	(47)	(47)	(47)
Histiocytic sarcoma	–	–	–	1 (2%)	–
Leukemia mononuclear	7 (15%)	11 (25%)	7 (15%)	12 (26%)	10 (21%)
Lymphoma malignant	–	–	1 (2%)	–	–
Mesothelioma malignant	–	–	–	1 (2%)	2 (4%)
Urethra	(0)	(0)	(0)	(0)	(1)
Urinary bladder	(48)	(43)	(48)	(47)	(47)
Leukemia mononuclear	–	–	–	3 (6%)	1 (2%)
Lymphoma malignant	–	–	1 (2%)	–	–
Mesothelioma malignant	–	–	–	–	1 (2%)
Transitional epithelium, papilloma	1 (2%)	–	–	–	–
Systemic Lesions					
Multiple organs	(48)[b]	(48)[b]	(48)[b]	(47)[b]	(48)[b]
Histiocytic sarcoma	1 (2%)	–	–	1 (2%)	1 (2%)
Leukemia mononuclear	21 (44%)	26 (54%)	27 (56%)	27 (57%)	31 (65%)
Lymphoma malignant	–	–	1 (2%)	–	–
Mesothelioma malignant	–	1 (2%)	4 (8%)	10 (21%)	17 (35%)
Neoplasm Summary					
Total animals with primary neoplasms[c]	48	47	48	47	47
Total primary neoplasms	148	131	159	158	171
Total animals with benign neoplasms	44	44	46	43	42
Total benign neoplasms	97	88	98	91	95
Total animals with malignant neoplasms	33	32	41	41	45
Total malignant neoplasms	51	43	60	66	76
Total animals with metastatic neoplasms	2	1	1	3	–
Total metastatic neoplasms	2	1	1	3	–
Total animals with neoplasms uncertain-benign or malignant	–	–	1	1	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Total uncertain neoplasms	–	–	1	1	–

[a]Number of animals examined microscopically at the site and the number of animals with neoplasm.
[b]Number of animals with any tissue examined microscopically.
[c]Primary neoplasms: all neoplasms except metastatic neoplasms.

Table A-2. Statistical Analysis of Neoplasms in Male Rats in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Adrenal Medulla: Benign Pheochromocytoma					
Overall rate[a]	10/46 (22%)	11/47 (23%)	14/48 (29%)	8/47 (17%)	9/48 (19%)
Adjusted rate[b]	26.4%	28.7%	37.8%	23.1%	30.6%
Terminal rate[c]	6/21 (29%)	4/18 (22%)	9/15 (60%)	2/7 (29%)	1/2 (50%)
First incidence (days)[d]	522	468	577	634	543
Poly-3 test[e]	P = 0.531	P = 0.513	P = 0.204	P = 0.478N	P = 0.457
Adrenal Medulla: Benign Pheochromocytoma, Bilateral					
Overall rate	2/46 (4%)	1/47 (2%)	1/48 (2%)	5/47 (11%)	2/48 (4%)
Adjusted rate	5.4%	2.7%	2.8%	14.7%	7.3%
Terminal rate	1/21 (5%)	0/18 (0%)	0/15 (0%)	1/7 (14%)	0/2 (0%)
First incidence (days)	725	574	730	676	554
Poly-3 test	P = 0.169	P = 0.502N	P = 0.508N	P = 0.180	P = 0.584
Adrenal Medulla: Malignant Pheochromocytoma					
Overall rate	4/46 (9%)	1/47 (2%)	4/48 (8%)	0/47 (0%)	2/48 (4%)
Adjusted rate	10.6%	2.8%	10.9%	0.0%	7.4%
Terminal rate	2/21 (10%)	0/18 (0%)	1/15 (7%)	0/7 (0%)	1/2 (50%)
First incidence (days)	522	715	639	–	662
Poly-3 test	P = 0.316N	P = 0.190N	P = 0.629	P = 0.076N	P = 0.502N
Brain (Cerebrum): Malignant Glioma					
Overall rate	1/48 (2%)	0/48 (0%)	0/48 (0%)	0/47 (0%)	0/48 (0%)
Adjusted rate	2.6%	0.0%	0.0%	0.0%	0.0%
Terminal rate	0/21 (0%)	0/18 (0%)	0/15 (0%)	0/7 (0%)	0/2 (0%)
First incidence (days)	725	–	–	–	–
Poly-3 test	P = 0.305N	P = 0.509N	P = 0.510N	P = 0.525N	P = 0.568N
Heart: Malignant Schwannoma					
Overall rate	2/48 (4%)	3/48 (6%)	3/48 (6%)	7/47 (15%)	8/48 (17%)
Adjusted rate	5.3%	8.3%	8.3%	19.6%	26.4%
Terminal rate	2/21 (10%)	2/18 (11%)	3/15 (20%)	1/7 (14%)	0/2 (0%)
First incidence (days)	736 (T)	715	737 (T)	550	439
Poly-3 test	P = 0.002**	P = 0.481	P = 0.478	P = 0.061	P = 0.015*

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Mammary Gland: Fibroadenoma					
Overall rate	4/45 (9%)	6/42 (14%)	3/45 (7%)	7/44 (16%)	6/42 (14%)
Adjusted rate	10.9%	18.3%	8.8%	21.3%	22.0%
Terminal rate	2/21 (10%)	4/15 (27%)	1/14 (7%)	1/7 (14%)	0/2 (0%)
First incidence (days)	702	674	449	632	446
Poly-3 test	P = 0.125	P = 0.297	P = 0.540N	P = 0.195	P = 0.197
Oral Mucosa: Squamous Cell Carcinoma					
Overall rate	1/48 (2%)	0/48 (0%)	1/48 (2%)	1/47 (2%)	0/48 (0%)
Adjusted rate	2.6%	0.0%	2.8%	3.0%	0.0%
Terminal rate	0/21 (0%)	0/18 (0%)	0/15 (0%)	0/7 (0%)	0/2 (0%)
First incidence (days)	725	–	729	634	–
Poly-3 test	P = 0.489N	P = 0.509N	P = 0.751	P = 0.735	P = 0.568N
Oral Mucosa: Squamous Cell Papilloma					
Overall rate	1/48 (2%)	1/48 (2%)	0/48 (0%)	2/47 (4%)	3/48 (6%)
Adjusted rate	2.6%	2.7%	0.0%	5.9%	10.9%
Terminal rate	1/21 (5%)	0/18 (0%)	0/15 (0%)	1/7 (14%)	0/2 (0%)
First incidence (days)	737 (T)	640	–	585	623
Poly-3 test	P = 0.050*	P = 0.754	P = 0.509N	P = 0.462	P = 0.201
Tongue: Squamous Cell Carcinoma					
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)	0/47 (0%)	0/48 (0%)
Adjusted rate	0.0%	0.0%	2.7%	0.0%	0.0%
Terminal rate	0/21 (0%)	0/18 (0%)	0/15 (0%)	0/7 (0%)	0/2 (0%)
First incidence (days)	–	–	497	–	–
Poly-3 test	P = 0.649N	–	P = 0.494	–	–
Tongue: Squamous Cell Papilloma					
Overall rate	0/48 (0%)	1/48 (2%)	0/48 (0%)	1/47 (2%)	4/48 (8%)
Adjusted rate	0.0%	2.7%	0.0%	3.0%	14.0%
Terminal rate	0/21 (0%)	0/18 (0%)	0/15 (0%)	1/7 (14%)	0/2 (0%)
First incidence (days)	–	648	–	737 (T)	516
Poly-3 test	P = 0.003**	P = 0.493	–	P = 0.475	P = 0.031*
Oral Mucosa and Tongue (Combined): Squamous Cell Carcinoma and Papilloma					
Overall rate	2/48 (4%)	2/48 (4%)	2/48 (4%)	3/47 (6%)	7/48 (15%)
Adjusted rate	5.3%	5.4%	5.4%	8.8%	23.9%
Terminal rate	1/21 (5%)	0/18 (0%)	0/15 (0%)	1/7 (14%)	0/2 (0%)
First incidence (days)	725	640	497	585	516
Poly-3 test	P = 0.005**	P = 0.686	P = 0.685	P = 0.454	P = 0.030*

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Pancreas: Acinar Cell Adenoma					
Overall rate	0/48 (0%)	1/44 (2%)	1/47 (2%)	0/46 (0%)	0/48 (0%)
Adjusted rate	0.0%	2.9%	2.8%	0.0%	0.0%
Terminal rate	0/21 (0%)	0/18 (0%)	1/15 (7%)	0/7 (0%)	0/2 (0%)
First incidence (days)	–	648	737 (T)	–	–
Poly-3 test	P = 0.435N	P = 0.484	P = 0.486	–	–
Pancreatic Islets: Adenoma					
Overall rate	4/48 (8%)	2/45 (4%)	2/48 (4%)	1/45 (2%)	1/48 (2%)
Adjusted rate	10.6%	5.7%	5.4%	3.1%	3.7%
Terminal rate	3/21 (14%)	0/18 (0%)	0/15 (0%)	0/7 (0%)	0/2 (0%)
First incidence (days)	733	640	577	577	723
Poly-3 test	P = 0.180N	P = 0.367N	P = 0.346N	P = 0.227N	P = 0.299N
Pancreatic Islets: Carcinoma					
Overall rate	1/48 (2%)	0/45 (0%)	0/48 (0%)	0/45 (0%)	0/48 (0%)
Adjusted rate	2.6%	0.0%	0.0%	0.0%	0.0%
Terminal rate	1/21 (5%)	0/18 (0%)	0/15 (0%)	0/7 (0%)	0/2 (0%)
First incidence (days)	737 (T)	–	–	–	–
Poly-3 test	P = 0.309N	P = 0.516N	P = 0.509N	P = 0.535N	P = 0.568N
Pancreatic Islets: Adenoma and Carcinoma (Combined)					
Overall rate	5/48 (10%)	2/45 (4%)	2/48 (4%)	1/45 (2%)	1/48 (2%)
Adjusted rate	13.2%	5.7%	5.4%	3.1%	3.7%
Terminal rate	4/21 (19%)	0/18 (0%)	0/15 (0%)	0/7 (0%)	0/2% (0%)
First incidence (days)	733	640	577	577	723
Poly-3 test	P = 0.114N	P = 0.240N	P = 0.222N	P = 0.138N	P = 0.200N
Pituitary Gland (Pars Distalis): Adenoma					
Overall rate	28/47 (60%)	23/46 (50%)	31/48 (65%)	21/47 (45%)	20/47 (43%)
Adjusted rate	69.1%	58.0%	75.0%	54.7%	60.5%
Terminal rate	14/20 (70%)	9/18 (50%)	11/15 (73%)	4/7 (57%)	2/2 (100%)
First incidence (days)	402	476	505	573	439
Poly-3 test	P = 0.204N	P = 0.197N	P = 0.352	P = 0.120N	P = 0.282N
Preputial Gland: Adenoma					
Overall rate	2/48 (4%)	0/48 (0%)	5/48 (10%)	3/47 (6%)	2/48 (4%)
Adjusted rate	5.3%	0.0%	13.7%	8.8%	7.4%
Terminal rate	2/21 (10%)	0/18 (0%)	3/15 (20%)	0/7 (0%)	0/2 0%)
First incidence (days)	736 (T)	–	652	676	660
Poly-3 test	P = 0.295	P = 0.246N	P = 0.197	P = 0.452	P = 0.568

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Preputial Gland: Carcinoma					
Overall rate	6/48 (13%)	1/48 (2%)	2/48 (4%)	6/47 (13%)	4/48 (8%)
Adjusted rate	15.8%	2.7%	5.4%	17.0%	13.7%
Terminal rate	4/21 (19%)	0/18 (0%)	0/15 (0%)	1/7 (14%)	0/2 (0%)
First incidence (days)	702	632	534	550	428
Poly-3 test	P = 0.275	P = 0.059N	P = 0.138N	P = 0.570	P = 0.542N
Preputial Gland: Squamous Cell Carcinoma					
Overall rate	6/48 (13%)	5/48 (10%)	4/48 (8%)	4/47 (9%)	3/48 (6%)
Adjusted rate	15.1%	13.1%	10.9%	11.6%	10.6%
Terminal rate	1/21 (5%)	0/18 (0%)	1/15 (7%)	1/7 (14%)	0/2 (0%)
First incidence (days)	527	466	577	585	534
Poly-3 test	P = 0.347N	P = 0.528N	P = 0.418N	P = 0.460N	P = 0.430N
Prostate: Adenoma					
Overall rate	1/47 (2%)	1/47 (2%)	0/48 (0%)	3/47 (6%)	1/48 (2%)
Adjusted rate	2.7%	2.8%	0.0%	8.9%	3.7%
Terminal rate	0/21 (0%)	0/18 (0%)	0/15 (0%)	1/7 (14%)	0/2 (0%)
First incidence (days)	733	558	–	682	723
Poly-3 test	P = 0.276	P = 0.752	P = 0.508N	P = 0.266	P = 0.680
Skin (Subcutaneous Tissue): Fibroma					
Overall rate	2/48 (4%)	0/48 (0%)	1/48 (2%)	1/47 (2%)	4/48 (8%)
Adjusted rate	5.3%	0.0%	2.8%	2.9%	14.1%
Terminal rate	2/21 (10%)	0/18 (0%)	0/15 (0%)	0/7 (0%)	0/2 (0%)
First incidence (days)	736 (T)	–	682	577	555
Poly-3 test	P = 0.043*	P = 0.246N	P = 0.515N	P = 0.538N	P = 0.215
Testes (Interstitial Cell): Adenoma					
Overall rate	33/48 (69%)	34/47 (72%)	33/48 (69%)	34/47 (72%)	27/48 (56%)
Adjusted rate	77.6%	80.5%	76.9%	80.5%	75.0%
Terminal rate	17/21 (81%)	15/18 (83%)	13/15 (87%)	7/7 (100%)	2/2 (100%)
First incidence (days)	486	466	497	487	428
Poly-3 test	P = 0.417N	P = 0.477	P = 0.576N	P = 0.474	P = 0.498N
Testes (Bilateral/Interstitial Cell): Adenoma					
Overall rate	22/48 (46%)	17/47 (36%)	17/48 (35%)	21/47 (45%)	10/48 (21%)
Adjusted rate	55.4%	44.3%	43.9%	56.0%	34.2%
Terminal rate	15/21 (71%)	11/18 (61%)	8/15 (53%)	5/7 (71%)	1/2 (50%)
First incidence (days)	564	514	549	556	518
Poly-3 test	P = 0.154N	P = 0.215N	P = 0.202N	P = 0.572	P = 0.056N

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Testes: Malignant Mesothelioma					
Overall rate	0/48 (0%)	1/47 (2%)	3/48 (6%)	6/47 (13%)	13/48 (27%)
Adjusted rate	0.0%	2.8%	8.3%	17.5%	40.5%
Terminal rate	0/21 (0%)	1/18 (6%)	1/15 (7%)	1/7 (14%)	0/2 (0%)
First incidence (days)	–	736 (T)	686	634	473
Poly-3 test	P < 0.001***	P = 0.490	P = 0.110	P = 0.010**	P < 0.001***
Epididymis: Malignant Mesothelioma					
Overall rate	0/48 (0%)	1/45 (2%)	3/48 (6%)	10/47 (21%)	17/47 (36%)
Adjusted rate	0.0%	2.9%	8.3%	28.2%	51.6%
Terminal rate	0/21 (0%)	1/18 (6%)	1/15 (7%)	2/7 (29%)	1/2 (50%)
First incidence (days)	–	736 (T)	686	556	473
Poly-3 test	P < 0.001***	P = 0.483	P = 0.110	P < 0.001***	P < 0.001***
Testes and Epididymis (Combined): Malignant Mesothelioma					
Overall rate	0/48 (0%)	1/48 (2%)	3/48 (6%)	10/47 (21%)	17/48 (35%)
Adjusted rate	0.0%	2.8%	8.3%	28.2%	51.4%
Terminal rate	0/21 (0%)	1/18 (6%)	1/15 (7%)	2/7 (29%)	1/2 (50%)
First incidence (days)	–	736 (T)	686	556	473
Poly-3 test	P < 0.001***	P = 0.491	P = 0.110	P < 0.001***	P < 0.001***
Thyroid Gland: C-Cell Adenoma					
Overall rate	4/47 (9%)	0/42 (0%)	1/48 (2%)	0/47 (0%)	1/46 (2%)
Adjusted rate	10.4%	0.0%	2.8%	0.0%	3.8%
Terminal rate	2/21 (10%)	0/18 (0%)	0/15 (0%)	0/7 (0%)	0/2 (0%)
First incidence (days)	481	–	725	–	439
Poly-3 test	P = 0.190N	P = 0.077N	P = 0.198N	P = 0.079N	P = 0.311N
Thyroid Gland: C-Cell Carcinoma					
Overall rate	1/47 (2%)	2/42 (5%)	1/48 (2%)	1/47 (2%)	0/46 (0%)
Adjusted rate	2.7%	5.8%	2.7%	3.0%	0.0%
Terminal rate	0/21 (0%)	0/18 (0%)	0/15 (0%)	0/7 (0%)	0/2 (0%)
First incidence (days)	708	624	518	676	–
Poly-3 test	P = 0.283N	P = 0.470	P = 0.757	P = 0.737	P = 0.572N
Thyroid Gland: Follicular Cell Adenoma					
Overall rate	2/47 (4%)	1/42 (2%)	3/48 (6%)	3/47 (6%)	8/46 (17%)
Adjusted rate	5.4%	3.0%	8.1%	8.9%	28.4%
Terminal rate	1/21 (5%)	1/18 (6%)	1/15 (7%)	2/7 (29%)	1/2 (50%)
First incidence (days)	724	737 (T)	549	635	520
Poly-3 test	P < 0.001***	P = 0.534N	P = 0.494	P = 0.454	P = 0.011*

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Thyroid Gland: Follicular Cell Carcinoma					
Overall rate	0/47 (0%)	2/42 (5%)	3/48 (6%)	1/47 (2%)	5/46 (11%)
Adjusted rate	0.0%	5.8%	8.2%	3.0%	18.3%
Terminal rate	0/21 (0%)	0/18 (0%)	2/15 (13%)	0/7 (0%)	0/2 (0%)
First incidence (days)	–	683	536	603	540
Poly-3 test	P = 0.014*	P = 0.217	P = 0.114	P = 0.481	P = 0.011*
Thyroid Gland: Follicular Cell Adenoma and Carcinoma (Combined)					
Overall rate	2/47 (4%)	3/42 (7%)	6/48 (13%)	4/47 (9%)	13/46 (28%)
Adjusted rate	5.4%	8.8%	16.0%	11.7%	43.8%
Terminal rate	1/21 (5%)	1/18 (6%)	3/15 (20%)	2/7 (29%)	1/2 (50%)
First incidence (days)	724	683	536	603	520
Poly-3 test	P < 0.001***	P = 0.460	P = 0.130	P = 0.295	P < 0.001***
Zymbal's Gland: Carcinoma					
Overall rate	0/48 (0%)	0/48 (0%)	3/48 (6%)	2/47 (4%)	3/48 (6%)
Adjusted rate	0.0%	0.0%	8.2%	5.8%	10.4%
Terminal rate	0/21 (0%)	0/18 (0%)	0/15 (0%)	0/7 (0%)	0/2 (0%)
First incidence (days)	–	–	602	418	473
Poly-3 test	P = 0.029*	–	P = 0.111	P = 0.216	P = 0.076
All Organs: Hemangioma					
Overall rate	0/48 (0%)	1/48 (2%)	0/48 (0%)	1/47 (2%)	0/48 (0%)
Adjusted rate	0.0%	2.8%	0.0%	2.9%	0.0%
Terminal rate	0/21 (0%)	1/18 (6%)	0/15 (0%)	0/7 (0%)	0/2 (0%)
First incidence (days)	–	737 (T)	–	573	–
Poly-3 test	P = 0.641	P = 0.491	–	P = 0.478	–
All Organs: Hemangiosarcoma or Hemangioma					
Overall rate	0/48 (0%)	1/48 (2%)	0/48 (0%)	1/47 (2%)	0/48 (0%)
Adjusted rate	0.0%	2.8%	0.0%	2.9%	0.0%
Terminal rate	0/21 (0%)	1/18 (6%)	0/15 (0%)	0/7 (0%)	0/2 (0%)
First incidence (days)	–	737 (T)	–	573	–
Poly-3 test	P = 0.641	P = 0.491	–	P = 0.478	–
All Organs: Histiocytic Sarcoma					
Overall rate	1/48 (2%)	0/48 (0%)	0/48 (0%)	1/47 (2%)	1/48 (2%)
Adjusted rate	2.6%	0.0%	0.0%	3.0%	3.7%
Terminal rate	0/21 (0%)	0/18 (0%)	0/15 (0%)	1/7 (14%)	0/2 (0%)
First incidence (days)	633	–	–	737 (T)	568
Poly-3 test	P = 0.333	P = 0.510N	P = 0.511N	P = 0.731	P = 0.680

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
All Organs: Leukemia (Mononuclear)					
Overall rate	21/48 (44%)	26/48 (54%)	27/48 (56%)	27/47 (57%)	31/48 (65%)
Adjusted rate	49.4%	60.2%	65.5%	65.3%	76.1%
Terminal rate	7/21 (33%)	7/18 (39%)	8/15 (53%)	3/7 (43%)	1/2 (50%)
First incidence (days)	481	468	505	484	410
Poly-3 test	P = 0.008**	P = 0.211	P = 0.093	P = 0.097	P = 0.006**
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, NOS, or Undifferentiated Cell Type)					
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)	0/47 (0%)	0/48 (0%)
Adjusted rate	0.0%	0.0%	2.8%	0.0%	0.0%
Terminal rate	0/21 (0%)	0/18 (0%)	0/15 (0%)	0/7 (0%)	0/2 (0%)
First incidence (days)	–	–	682	–	–
Poly-3 test	P = 0.650N	–	P = 0.492	–	–
All Organs: Mesothelioma (Benign, Malignant, NOS)					
Overall rate	0/48 (0%)	1/48 (2%)	4/48 (8%)	10/47 (21%)	17/48 (35%)
Adjusted rate	0.0%	2.8%	11.0%	28.2%	51.4%
Terminal rate	0/21 (0%)	1/18 (6%)	2/15 (13%)	2/7 (29%)	1/2 (50%)
First incidence (days)	–	736 (T)	686	556	473
Poly-3 test	P < 0.001***	P = 0.491	P = 0.054	P < 0.001***	P < 0.001***
All Organs: Osteoma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/47 (2%)	1/48 (2%)
Adjusted rate	0.0%	0.0%	0.0%	3.0%	3.7%
Terminal rate	0/21 (0%)	0/18 (0%)	0/15 (0%)	1/7 (14%)	0/2 (0%)
First incidence (days)	–	–	–	737 (T)	676
Poly-3 test	P = 0.098	–	–	P = 0.475	P = 0.434
All Organs: Osteosarcoma					
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)	1/47 (2%)	0/48 (0%)
Adjusted rate	0.0%	0.0%	2.7%	3.0%	0.0%
Terminal rate	0/21 (0%)	0/18 (0%)	0/15 (0%)	0/7 (0%)	0/2 (0%)
First incidence (days)	–	–	506	634	–
Poly-3 test	P = 0.524	–	P = 0.494	P = 0.477	–
All Organs: Osteosarcoma or Osteoma					
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)	2/47 (4%)	1/48 (2%)
Adjusted rate	0.0%	0.0%	2.7%	5.9%	3.7%
Terminal rate	0/21 (0%)	0/18 (0%)	0/15 (0%)	1/7 (14%)	0/2 (0%)
First incidence (days)	–	–	506	634	676
Poly-3 test	P = 0.124	–	P = 0.494	P = 0.212	P = 0.434

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
All Organs: Benign Neoplasms					
Overall rate	44/48 (92%)	44/48 (92%)	46/48 (96%)	43/47 (92%)	42/48 (88%)
Adjusted rate	95.0%	95.8%	97.7%	95.8%	95.5%
Terminal rate	20/21 (95%)	17/18 (94%)	15/15 (100%)	7/7 (100%)	2/2 (100%)
First incidence (days)	402	466	449	487	428
Poly-3 test	P = 0.589N	P = 0.637	P = 0.430	P = 0.645	P = 0.685
All Organs: Malignant Neoplasms					
Overall rate	33/48 (69%)	32/48 (67%)	41/48 (85%)	41/47 (87%)	45/48 (94%)
Adjusted rate	73.1%	70.7%	89.7%	89.2%	97.4%
Terminal rate	12/21 (57%)	8/18 (44%)	13/15 (87%)	6/7 (86%)	2/2 (100%)
First incidence (days)	481	466	497	418	410
Poly-3 test	P < 0.001***	P = 0.488N	P = 0.030*	P = 0.037*	P < 0.001***
All Organs: Benign or Malignant Neoplasms					
Overall rate	48/48 (100%)	47/48 (98%)	48/48 (100%)	47/47 (100%)	47/48 (98%)
Adjusted rate	100.0%	99.8%	100.0%	100.0%	99.7%
Terminal rate	21/21 (100%)	18/18 (100%)	15/15 (100%)	7/7 (100%)	2/2 (100%)
First incidence (days)	402	466	449	418	410
Poly-3 test	P = 0.998N	P = 1.000N	–	–	P = 1.000N

[a]Number of animals with neoplasm per number of animals examined microscopically.
[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c]Observed incidence at the terminal sacrifice.
[d]T indicates terminal sacrifice.
[e]Beneath the 0 mM Glycidamide are the p-values associated with the trend test. Beneath the treated (0.0875, 0.175, 0.35, and 0.70 mM Glycidamide) group incidences are the p-values corresponding to pairwise comparisons between the 0 mM Glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.

Table A-3. Historical Incidence of Thyroid Gland Follicular Cell Neoplasms in NCTR Control Male F344/N Nctr Rats

Study (Report Date)	Route of Administration	Incidence in Controls		
		Adenoma	Carcinoma	Adenoma or Carcinoma
Sulfamethazine (February 1988)	Diet	0/170 (0.0%)	0/170 (0.0%)	0/170 (0.0%)
Gentian Violet (November 1988)	Diet	1/163 (0.6%)	0/163 (0.0%)	1/163 (0.6%)
Doxylamine (April 1991)	Diet	1/48 (2.1%)	0/48 (0.0%)	1/48 (2.1%)
Triprolidine (June 1991)	Diet	0/40 (0.0%)	0/40 (0.0%)	0/40 (0.0%)
Pyrilamine (July 1991)	Diet	0/42 (0.0%)	0/42 (0.0%)	0/42 (0.0%)
Fumonisin B_1 (March 1999)	Diet	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Leucomalachite Green (June 2001)	Diet	0/47 (0.0%)	0/47 (0.0%)	0/47 (0.0%)
Acrylamide (February 2012)	Drinking water	0/47 (0.0%)	1/47 (2.1%)	1/47 (2.1%)
Aloe vera Whole Leaf (2013)	Drinking water	2/48 (4.2%)	0/48 (0.0%)	2/48 (4.2%)
Total (%) (All studies)		4/653 (0.6%)	1/653 (0.2%)	5/653 (0.8%)
Range		0.0–4.2%	0.0–2.1%	0.0–4.2%
Total (%) (Drinking water studies)		2/95 (2.1%)	1/95 (1.1%)	3/95 (3.2%)
Range		0.0–4.2%	0.0–2.1%	2.1–4.2%

Table A-4. Historical Incidence of Malignant Mesothelioma (All Sites) in NCTR Control Male F344/N Nctr Rats

Study (Report Date)	Route of Administration	Incidence in Controls
Sulfamethazine (February 1988)	Diet	11/179 (6.1%)
Gentian Violet (November 1988)	Diet	6/180 (3.3%)
Doxylamine (April 1991)	Diet	2/48 (4.2%)
Triprolidine (June 1991)	Diet	3/47 (6.4%)
Pyrilamine (July 1991)	Diet	2/48 (4.2%)
Fumonisin B_1 (March 1999)	Diet	3/48 (6.3%)
Leucomalachite Green (June 2001)	Diet	2/48 (4.2%)
Acrylamide (February 2012)	Drinking water	2/48 (4.2%)
Aloe vera Whole Leaf (2013)	Drinking water	0/48 (0.0%)
Total (%) (All studies)		31/694 (4.5%)
Range		0.0–6.4%
Total (%) (Drinking water studies)		2/96 (2.1%)
Range		0.0–4.2%

Table A-5. Historical Incidence of Malignant Schwannoma of the Heart in NCTR Control Male F344/N Nctr Rats

Study (Report Date)	Route of Administration	Incidence in Controls
Sulfamethazine (February 1988)	Diet	0/179 (0.0%)
Gentian Violet (November 1988)	Diet	0/179 (0.0%)
Doxylamine (April 1991)	Diet	0/48 (0.0%)
Triprolidine (June 1991)	Diet	0/47 (0.0%)
Pyrilamine (July 1991)	Diet	0/48 (0.0%)
Fumonisin B_1 (March 1999)	Diet	0/48 (0.0%)
Leucomalachite Green (June 2001)	Diet	0/48 (0.0%)
Acrylamide (February 2012)	Drinking water	1/48 (2.1%)
Aloe vera Whole Leaf (2013)	Drinking water	1/48 (2.1%)
Total (%) (All studies)		2/693 (0.3%)
Range		0.0–2.1%
Total (%) (Drinking water studies)		2/96 (2.1%)
Range		2.1%

Table A-6. Historical Incidence of Mononuclear Cell Leukemia in NCTR Control Male F344/N Nctr Rats

Study (Report Date)	Route of Administration	Incidence in Controls
Sulfamethazine (February 1988)	Diet	62/179 (34.6%)
Gentian Violet (November 1988)	Diet	104/180 (57.8%)
Doxylamine (April 1991)	Diet	18/48 (37.5%)
Triprolidine (June 1991)	Diet	17/47 (36.2%)
Pyrilamine (July 1991)	Diet	15/48 (31.3%)
Fumonisin B_1 (March 1999)	Diet	24/48 (50.0%)
Leucomalachite Green (June 2001)	Diet	29/48 (60.4%)
Acrylamide (February 2012)	Drinking water	31/48 (64.6%)
Aloe vera Whole Leaf (2013)	Drinking water	27/48 (56.3%)
Total (%) (All studies)		327/694 (47.1%)
Range		31.3–64.6%
Total (%) (Drinking water studies)		58/96 (60.4%)
Range		56.3–64.6%

Table A-7. Historical Incidence of Neoplasms of the Oral Cavity in NCTR Control Male F344/N Nctr Rats

Study (Report Date)	Route of Administration	Incidence in Controls		
		Papilloma or Squamous Cell Papilloma	Squamous Cell Carcinoma	Papilloma, Squamous Cell Papilloma, or Squamous Cell Carcinoma
Sulfamethazine (February 1988)	Diet	0/179 (0.0%)	0/179 (0.0%)	0/179 (0.0%)
Gentian Violet (November 1988)	Diet	0/178 (0.0%)	1/178 (0.6%)	1/178 (0.6%)
Doxylamine (April 1991)	Diet	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Triprolidine (June 1991)	Diet	1/47 (2.1%)	0/47 (0.0%)	1/47 (2.1%)
Pyrilamine (July 1991)	Diet	–[a]	–[a]	–[a]
Fumonisin B_1 (March 1999)	Diet	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Leucomalachite Green (June 2001)	Diet	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Acrylamide (February 2012)	Drinking water	1/48 (2.1%)	0/48 (0.0%)	1/48 (2.1%)
Aloe vera Whole Leaf (2013)	Drinking water	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Total (%) (All studies)		2/644 (0.3%)	1/644 (0.2%)	3/644 (0.5%)
Range		0.0–2.1%	0.0–0.6%	0.0–2.1%
Total (%) (Drinking water studies)		1/96 (1.0%)	0/96 (0.0%)	1/96 (1.0%)
Range		0.0–2.1%	0.0%	0.0–2.1%

[a]Not reported.

Table A-8. Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Two-year Drinking Water Study of Glycidamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Disposition Summary					
Animals initially in study	48	48	48	48	48
Early deaths					
Moribund sacrifice	16	13	24	26	36
Natural deaths	4	10	4	2	1
Survivors					
Moribund sacrifice	7	5	4	10	7
Natural deaths	–	2	1	2	2
Terminal sacrifice	21	18	15	7	2
Other	–	–	–	1	–
Animals examined microscopically	48	48	48	47	48
Alimentary System					
Esophagus	(48)	(47)	(48)	(47)	(48)
Inflammation, suppurative	–	–	–	1 (2%)	–
Ulcer	–	–	–	1 (2%)	–
Intestine large, cecum	(45)	(37)	(43)	(42)	(46)
Hyperplasia, lymphoid	–	–	–	1 (2%)	–
Lumen, dilatation	–	–	–	1 (2%)	–
Intestine large, colon	(46)	(38)	(44)	(43)	(45)
Hyperplasia, lymphoid	1 (2%)	–	–	–	–
Intestine large, rectum	(46)	(37)	(44)	(44)	(45)
Edema	1 (2%)	–	–	–	–
Intestine small, duodenum	(46)	(37)	(43)	(43)	(46)
Inflammation, chronic active	–	1 (3%)	–	–	–
Necrosis	–	1 (3%)	–	–	–
Epithelium, hyperplasia	–	1 (3%)	–	1 (2%)	–
Intestine small, ileum	(45)	(37)	(43)	(43)	(45)
Hyperplasia, lymphoid	–	–	–	–	2 (4%)
Epithelium, hyperplasia	–	–	–	1 (2%)	–
Intestine small, jejunum	(45)	(36)	(43)	(43)	(45)
Bacterium	–	1 (3%)	–	–	–
Hyperplasia, lymphoid	–	–	–	–	1 (2%)
Inflammation, chronic active	–	1 (3%)	–	–	–
Necrosis	–	1 (3%)	–	–	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Liver	(47)	(47)	(48)	(47)	(47)
Angiectasis	1 (2%)	–	–	–	1 (2%)
Basophilic focus	6 (13%)	1 (2%)	6 (13%)	7 (15%)	2 (4%)
Basophilic focus, multiple	10 (21%)	8 (17%)	5 (10%)	1 (2%)	2 (4%)
Clear cell focus	2 (4%)	2 (4%)	–	–	1 (2%)
Congestion	–	–	1 (2%)	1 (2%)	1 (2%)
Deformity	3 (6%)	4 (9%)	3 (6%)	4 (9%)	3 (6%)
Degeneration, cystic	16 (34%)	20 (43%)	21 (44%)	13 (28%)	21 (45%)
Eosinophilic focus	10 (21%)	6 (13%)	9 (19%)	5 (11%)	3 (6%)
Eosinophilic focus, multiple	7 (15%)	5 (11%)	5 (10%)	2 (4%)	2 (4%)
Granuloma	1 (2%)	–	–	–	–
Hemorrhage	–	–	1 (2%)	–	–
Hepatodiaphragmatic nodule	–	1 (2%)	–	1 (2%)	1 (2%)
Hypertrophy	–	1 (2%)	–	–	–
Infiltration cellular, lymphocyte	–	–	–	1 (2%)	1 (2%)
Infiltration cellular, polymorphonuclear	–	1 (2%)	–	–	–
Inflammation, chronic active	7 (15%)	5 (11%)	7 (15%)	4 (9%)	2 (4%)
Mineralization	–	–	–	1 (2%)	–
Mixed cell focus	1 (2%)	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Necrosis	1 (2%)	5 (11%)	2 (4%)	7 (15%)	5 (11%)
Pigmentation	3 (6%)	5 (11%)	6 (13%)	5 (11%)	3 (6%)
Thrombosis	1 (2%)	2 (4%)	–	–	2 (4%)
Vacuolization cytoplasmic	17 (36%)	15 (32%)	14 (29%)	12 (26%)	12 (26%)
Bile duct, hyperplasia	39 (83%)	38 (81%)	39 (81%)	38 (81%)	36 (77%)
Biliary tract, fibrosis	–	1 (2%)	1 (2%)	1 (2%)	–
Hepatocyte, degeneration	2 (4%)	6 (13%)	6 (13%)	10 (21%)	8 (17%)
Hepatocyte, hyperplasia	1 (2%)	1 (2%)	2 (4%)	–	–
Oval cell, hyperplasia	–	–	1 (2%)	–	1 (2%)
Mesentery	(3)	(8)	(6)	(9)	(4)
Hemorrhage	–	–	1 (17%)	–	–
Pigmentation	–	–	1 (17%)	–	–
Fat, necrosis	2 (67%)	6 (75%)	6 (100%)	7 (78%)	1 (25%)
Oral Mucosa	(4)	(3)	(2)	(6)	(7)
Foreign body	–	–	1 (50%)	–	–
Keratin cyst	–	–	–	1 (17%)	1 (14%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Epithelium, hyperplasia	2 (50%)	2 (67%)	1 (50%)	3 (50%)	2 (29%)
Epithelium, hyperplasia, basal cell	–	–	–	–	1 (14%)
Pancreas	(48)	(44)	(47)	(46)	(48)
Angiectasis	–	–	–	–	1 (2%)
Inflammation, chronic active	1 (2%)	–	–	–	–
Polyarteritis	–	1 (2%)	–	–	–
Vacuolization cytoplasmic	–	–	1 (2%)	–	–
Acinar cell, hyperplasia	–	–	1 (2%)	1 (2%)	–
Acinus, degeneration	25 (52%)	30 (68%)	19 (40%)	25 (54%)	23 (48%)
Salivary glands	(48)	(44)	(48)	(46)	(47)
Stomach, Forestomach	(46)	(46)	(46)	(47)	(47)
Edema	1 (2%)	–	–	–	–
Inflammation, chronic active	–	3 (7%)	2 (4%)	3 (6%)	–
Ulcer	–	3 (7%)	2 (4%)	5 (11%)	–
Epithelium, hyperplasia	4 (9%)	6 (13%)	3 (7%)	9 (19%)	2 (4%)
Stomach, glandular	(46)	(38)	(44)	(44)	(46)
Edema	1 (2%)	–	–	–	–
Inflammation, suppurative	–	–	1 (2%)	–	–
Inflammation, chronic active	2 (4%)	–	3 (7%)	1 (2%)	–
Necrosis	5 (11%)	3 (8%)	2 (5%)	2 (5%)	4 (9%)
Pigmentation	1 (2%)	2 (5%)	1 (2%)	1 (2%)	1 (2%)
Ulcer	1 (2%)	1 (3%)	2 (5%)	–	1 (2%)
Epithelium, degeneration	–	–	1 (2%)	1 (2%)	–
Epithelium, hyperplasia	2 (4%)	–	–	–	1 (2%)
Tongue	(0)	(1)	(1)	(2)	(6)
Inflammation, suppurative	–	1 (100%)	–	–	–
Epithelium, hyperplasia	–	–	–	1 (50%)	–
Cardiovascular System					
Blood vessel	(48)	(48)	(48)	(47)	(48)
Heart	(48)	(48)	(48)	(47)	(48)
Cardiomyopathy	37 (77%)	37 (77%)	36 (75%)	31 (66%)	34 (71%)
Congestion	–	–	–	1 (2%)	1 (2%)
Metaplasia, osseous	–	1 (2%)	–	–	2 (4%)
Mineralization	1 (2%)	2 (4%)	1 (2%)	–	–
Pigmentation	1 (2%)	–	–	–	–
Thrombosis	10 (21%)	14 (29%)	14 (29%)	8 (17%)	9 (19%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Atrium, dilatation	2 (4%)	2 (4%)	1 (2%)	–	2 (4%)
Ventricle, dilatation	–	–	2 (4%)	2 (4%)	1 (2%)
Endocrine System					
Adrenal cortex	(47)	(47)	(48)	(47)	(47)
Accessory adrenal cortical nodule	–	–	1 (2%)	3 (6%)	
Atrophy	–	–	–	–	1 (2%)
Degeneration, cystic	–	1 (2%)	–	–	1 (2%)
Hyperplasia	3 (6%)	–	–	1 (2%)	–
Hypertrophy	1 (2%)	–	2 (4%)	1 (2%)	3 (6%)
Metaplasia, osseous	–	–	1 (2%)	1 (2%)	–
Thrombosis	–	–	–	–	1 (2%)
Vacuolization cytoplasmic	26 (55%)	27 (57%)	31 (65%)	28 (60%)	34 (72%)
Adrenal Medulla	(46)	(47)	(48)	(47)	(48)
Hyperplasia	13 (28%)	11 (23%)	7 (15%)	5 (11%)	6 (13%)
Necrosis	–	1 (2%)	–	–	–
Islets, pancreatic	(48)	(45)	(48)	(45)	(48)
Fibrosis	–	1 (2%)	–	–	–
Hyperplasia	–	4 (9%)	2 (4%)	2 (4%)	3 (6%)
Parathyroid gland	(48)	(47)	(47)	(45)	(48)
Hyperplasia	5 (10%)	10 (21%)	6 (13%)	3 (7%)	7 (15%)
Pituitary gland	(47)	(46)	(48)	(47)	(47)
Hemorrhage	–	–	–	1 (2%)	–
Inflammation, suppurative	–	–	–	1 (2%)	–
Pars distalis, angiectasis	1 (2%)	1 (2%)	–	–	–
Pars distalis, cyst	2 (4%)	–	3 (6%)	1 (2%)	3 (6%)
Pars Distalis, hyperplasia	10 (21%)	10 (22%)	6 (13%)	10 (21%)	8 (17%)
Pars intermedia, cyst	–	1 (2%)	–	–	–
Pars nervosa, Rathke's cleft, degeneration	–	2 (4%)	–	–	–
Thyroid gland	(47)	(42)	(48)	(47)	(46)
Cyst	1 (2%)	1 (2%)	1 (2%)	3 (6%)	–
C-cell, hyperplasia	14 (30%)	16 (38%)	18 (38%)	18 (38%)	14 (30%)
Follicular cell, hyperplasia	–	2 (5%)	3 (6%)	–	4 (9%)
General Body System					
Tissue NOS	(2)	(0)	(1)	(1)	(1)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Genital System					
Coagulating gland	(0)	(0)	(0)	(0)	(1)
Inflammation, suppurative	–	–	–	–	1 (100%)
Necrosis	–	–	–	–	1 (100%)
Epithelium, hyperplasia	–	–	–	–	1 (100%)
Epididymis	(48)	(45)	(48)	(47)	(47)
Exfoliated germ cell	–	1 (2%)	2 (4%)	3 (6%)	4 (9%)
Hypospermia	36 (75%)	29 (64%)	31 (65%)	31 (66%)	24 (51%)
Epithelium, degeneration	31 (65%)	25 (56%)	27 (56%)	28 (60%)	18 (38%)
Preputial gland	(48)	(48)	(48)	(47)	(48)
Cyst	–	–	1 (2%)	–	1 (2%)
Fibrosis	–	–	–	–	2 (4%)
Hemorrhage	1 (2%)	–	–	–	–
Hyperkeratosis	–	–	–	1 (2%)	2 (4%)
Infiltration cellular, plasma cell	–	1 (2%)	–	–	–
Inflammation, suppurative	21 (44%)	27 (56%)	25 (52%)	24 (51%)	18 (38%)
Inflammation, chronic active	14 (29%)	7 (15%)	9 (19%)	8 (17%)	10 (21%)
Necrosis	1 (2%)	–	–	1 (2%)	1 (2%)
Acinus, degeneration	24 (50%)	25 (52%)	25 (52%)	26 (55%)	22 (46%)
Duct, ectasia	25 (52%)	24 (50%)	24 (50%)	23 (49%)	17 (35%)
Epithelium, hyperplasia	–	–	2 (4%)	–	2 (4%)
Prostate	(47)	(47)	(48)	(47)	(48)
Cyst multilocular	1 (2%)	–	–	–	2 (4%)
Infiltration cellular, lymphocyte	–	–	1 (2%)	–	–
Inflammation, suppurative	23 (49%)	24 (51%)	29 (60%)	21 (45%)	22 (46%)
Inflammation, chronic active	11 (23%)	6 (13%)	8 (17%)	8 (17%)	7 (15%)
Necrosis	–	1 (2%)	–	–	1 (2%)
Acinus, degeneration	1 (2%)	–	–	1 (2%)	–
Epithelium, hyperplasia	8 (17%)	7 (15%)	6 (13%)	8 (17%)	3 (6%)
Seminal vesicle	(47)	(38)	(46)	(46)	(47)
Atrophy	27 (57%)	17 (45%)	25 (54%)	27 (59%)	18 (38%)
Inflammation, suppurative	–	–	–	–	1 (2%)
Necrosis	–	–	–	–	1 (2%)
Epithelium, hyperplasia	–	–	–	–	2 (4%)
Lumen, dilatation	2 (4%)	1 (3%)	3 (7%)	1 (2%)	–
Testes	(48)	(47)	(48)	(47)	(48)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Cyst	1 (2%)	–	–	–	–
Polyarteritis	2 (4%)	–	1 (2%)	–	–
Interstitial cell, hyperplasia	7 (15%)	3 (6%)	8 (17%)	6 (13%)	11 (23%)
Seminiferous tubule, degeneration	38 (79%)	35 (74%)	36 (75%)	38 (81%)	41 (85%)
Hematopoietic System					
Bone marrow	(47)	(40)	(45)	(46)	(46)
Atrophy	–	4 (10%)	1 (2%)	1 (2%)	–
Hyperplasia	6 (13%)	4 (10%)	10 (22%)	12 (26%)	10 (22%)
Lymph node	(17)	(19)	(19)	(23)	(23)
Inguinal, hyperplasia, lymphoid	–	–	–	1 (4%)	–
Inguinal, infiltration cellular, plasma cell	–	–	–	1 (4%)	–
Lumbar, hyperplasia, lymphoid	–	–	–	1 (4%)	1 (4%)
Lumbar, infiltration cellular, plasma cell	–	–	1 (5%)	–	1 (4%)
Lumbar, sinus, dilatation	–	2 (11%)	2 (11%)	1 (4%)	2 (9%)
Mediastinal, hemorrhage	–	1 (5%)	–	2 (9%)	2 (9%)
Mediastinal, hyperplasia, lymphoid	–	–	–	–	1 (4%)
Mediastinal, pigmentation	–	–	1 (5%)	–	–
Mediastinal, sinus, dilatation	1 (6%)	2 (11%)	3 (16%)	2 (9%)	3 (13%)
Pancreatic, hemorrhage	–	–	–	–	1 (4%)
Pancreatic, hyperplasia, lymphoid	1 (6%)	1 (5%)	1 (5%)	1 (4%)	1 (4%)
Pancreatic, infiltration cellular, plasma cell	1 (6%)	–	–	–	–
Pancreatic, sinus, dilatation	–	1 (5%)	1 (5%)	2 (9%)	2 (9%)
Renal, hemorrhage	–	1 (5%)	–	–	–
Renal, hyperplasia, lymphoid	1 (6%)	–	–	–	2 (9%)
Renal, infiltration cellular, histiocyte	1 (6%)	–	–	–	
Renal, infiltration cellular, plasma cell	–	–	3 (16%)	–	–
Renal, pigmentation	1 (6%)	–	–	–	1 (4%)
Renal, sinus, dilatation	5 (29%)	1 (5%)	5 (26%)	2 (9%)	3 (13%)
Sinus, dilatation	–	1 (5%)	–	–	–
Lymph node, mandibular	(48)	(45)	(48)	(47)	(47)
Hemorrhage	1 (2%)	1 (2%)	1 (2%)	1 (2%)	–
Hyperplasia, lymphoid	7 (15%)	6 (13%)	6 (13%)	7 (15%)	11 (23%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Infiltration cellular, plasma cell	27 (56%)	21 (47%)	21 (44%)	20 (43%)	19 (40%)
Infiltration cellular, polymorphonuclear	–	1 (2%)	–	–	–
Sinus, dilatation	15 (31%)	10 (22%)	9 (19%)	6 (13%)	6 (13%)
Lymph node, mesenteric	(48)	(46)	(46)	(46)	(47)
Angiectasis	–	–	1 (2%)	–	–
Hemorrhage	–	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Hyperplasia, lymphoid	15 (31%)	9 (20%)	11 (24%)	12 (26%)	10 (21%)
Infiltration cellular, histiocyte	–	–	1 (2%)	–	1 (2%)
Infiltration cellular, plasma cell	8 (17%)	6 (13%)	2 (4%)	1 (2%)	1 (2%)
Metaplasia, osseous	–	–	1 (2%)	–	–
Sinus, dilatation	3 (6%)	2 (4%)	2 (4%)	5 (11%)	5 (11%)
Spleen	(48)	(47)	(48)	(47)	(47)
Accessory spleen	1 (2%)	3 (6%)	–	1 (2%)	3 (6%)
Congestion	3 (6%)	3 (6%)	3 (6%)	2 (4%)	2 (4%)
Depletion lymphoid	–	–	1 (2%)	1 (2%)	1 (2%)
Developmental malformation	1 (2%)	–	–	–	–
Fibrosis	6 (13%)	10 (21%)	13 (27%)	13 (28%)	11 (23%)
Hematopoietic cell proliferation	4 (8%)	4 (9%)	6 (13%)	8 (17%)	7 (15%)
Hemorrhage	–	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Hyperplasia, lymphoid	3 (6%)	2 (4%)	2 (4%)	–	1 (2%)
Hyperplasia, stromal	–	–	1 (2%)	–	1 (2%)
Necrosis	1 (2%)	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Pigmentation	19 (40%)	17 (36%)	15 (31%)	13 (28%)	9 (19%)
Red pulp, hyperplasia	1 (2%)	–	–	–	–
Thymus	(43)	(38)	(43)	(42)	(43)
Atrophy	40 (93%)	37 (97%)	41 (95%)	34 (81%)	40 (93%)
Hemorrhage	–	1 (3%)	–	–	–
Integumentary System					
Mammary gland	(45)	(42)	(45)	(44)	(42)
Fibrosis	–	–	–	1 (2%)	1 (2%)
Galactocele	4 (9%)	4 (10%)	5 (11%)	4 (9%)	6 (14%)
Lactation	12 (27%)	8 (19%)	17 (38%)	6 (14%)	7 (17%)
Alveolus, hyperplasia	2 (4%)	1 (2%)	4 (9%)	1 (2%)	1 (2%)
Duct, dilatation	1 (2%)	–	–	–	–
Skin	(48)	(48)	(48)	(47)	(48)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Cyst epithelial inclusion	3 (6%)	1 (2%)	3 (6%)	5 (11%)	4 (8%)
Fibrosis	2 (4%)	1 (2%)	–	2 (4%)	2 (4%)
Hyperkeratosis	1 (2%)	2 (4%)	–	1 (2%)	–
Infiltration cellular, plasma cell	1 (2%)	–	–	–	–
Inflammation, suppurative	–	–	–	1 (2%)	–
Inflammation, chronic	–	–	–	1 (2%)	–
Inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)	–	1 (2%)
Necrosis	1 (2%)	–	–	–	1 (2%)
Ulcer	1 (2%)	–	–	–	–
Epithelium, hyperplasia	–	2 (4%)	–	1 (2%)	1 (2%)
Sebaceous gland, hyperplasia	–	–	–	–	1 (2%)
Musculoskeletal System					
Bone	(1)	(0)	(2)	(3)	(1)
Cranium, deformity	–	–	1 (50%)	1 (33%)	–
Vertebra, fracture	1 (100%)	–	–	–	–
Bone, femur	(48)	(48)	(48)	(47)	(48)
Fibrous osteodystrophy	–	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Osteopetrosis	–	2 (4%)	–	1 (2%)	–
Skeletal muscle	(48)	(48)	(48)	(47)	(48)
Nervous System					
Brain, brain stem	(48)	(48)	(48)	(47)	(48)
Compression	15 (31%)	10 (21%)	13 (27%)	8 (17%)	9 (19%)
Gliosis	–	1 (2%)	–	–	3 (6%)
Hemorrhage	1 (2%)	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Necrosis	–	1 (2%)	–	–	–
Brain, cerebellum	(48)	(48)	(48)	(47)	(48)
Hemorrhage	–	–	1 (2%)	1 (2%)	1 (2%)
Infiltration cellular, polymorphonuclear	–	–	–	1 (2%)	–
Ventricle, dilatation	2 (4%)	1 (2%)	3 (6%)	1 (2%)	–
Brain, cerebrum	(48)	(48)	(48)	(47)	(48)
Compression	–	–	–	1 (2%)	–
Gliosis	–	–	–	–	2 (4%)
Hemorrhage	–	–	1 (2%)	–	1 (2%)
Mineralization	–	–	2 (4%)	1 (2%)	–
Meninges, hemorrhage	1 (2%)	–	–	–	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Ventricle, dilatation	6 (13%)	2 (4%)	4 (8%)	3 (6%)	–
Peripheral nerve, sciatic	(48)	(48)	(48)	(47)	(48)
Axon, degeneration	31 (65%)	22 (46%)	26 (54%)	28 (60%)	28 (58%)
Spinal cord, cervical	(47)	(46)	(48)	(47)	(48)
Cyst	–	–	1 (2%)	–	–
Gliosis	–	–	–	–	1 (2%)
Hemorrhage	2 (4%)	–	1 (2%)	1 (2%)	–
Mineralization	–	–	–	–	1 (2%)
Axon, degeneration	25 (53%)	19 (41%)	23 (48%)	28 (60%)	27 (56%)
Spinal cord, lumbar	(47)	(46)	(48)	(47)	(48)
Gliosis	1 (2%)	–	–	–	–
Mineralization	–	–	–	–	2 (4%)
Axon, degeneration	5 (11%)	7 (15%)	4 (8%)	2 (4%)	5 (10%)
Nerve, degeneration	36 (77%)	26 (57%)	29 (60%)	32 (68%)	26 (54%)
Nerve, gliosis	2 (4%)	2 (4%)	–	–	1 (2%)
Spinal cord, thoracic	(47)	(46)	(48)	(47)	(48)
Hemorrhage	–	–	–	1 (2%)	–
Mineralization	–	–	–	–	1 (2%)
Axon, degeneration	23 (49%)	22 (48%)	25 (52%)	30 (64%)	27 (56%)
Nerve, degeneration	5 (11%)	8 (17%)	3 (6%)	4 (9%)	5 (10%)
Respiratory System					
Lung	(48)	(47)	(48)	(47)	(47)
Congestion	1 (2%)	–	–	–	–
Foreign body	–	–	–	–	1 (2%)
Hemorrhage	2 (4%)	–	–	1 (2%)	2 (4%)
Infiltration cellular, histiocyte	12 (25%)	4 (9%)	8 (17%)	5 (11%)	15 (32%)
Infiltration cellular, lymphocyte	–	–	–	–	1 (2%)
Inflammation, suppurative	–	–	1 (2%)	–	–
Inflammation, granulomatous	–	–	–	1 (2%)	1 (2%)
Inflammation, chronic	–	–	–	1 (2%)	–
Mineralization	–	–	1 (2%)	–	–
Pigmentation	2 (4%)	2 (4%)	3 (6%)	–	2 (4%)
Alveolar epithelium, hyperplasia	1 (2%)	1 (2%)	1 (2%)	1 (2%)	5 (11%)
Bronchus, inflammation, suppurative	–	–	–	1 (2%)	–
Endothelium, hyperplasia	–	–	–	–	1 (2%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Nose	(48)	(45)	(47)	(45)	(48)
Inflammation, suppurative	3 (6%)	5 (11%)	1 (2%)	4 (9%)	2 (4%)
Inflammation, chronic active	1 (2%)	2 (4%)	–	–	1 (2%)
Goblet cell, hyperplasia	–	1 (2%)	–	1 (2%)	2 (4%)
Nasopharyngeal duct, hyperkeratosis	–	–	–	1 (2%)	–
Nasopharyngeal duct, inflammation, suppurative	–	–	–	1 (2%)	–
Trachea	(48)	(43)	(48)	(47)	(47)
Special Senses System					
Ear	(0)	(0)	(1)	(1)	(0)
Eye	(45)	(38)	(43)	(44)	(45)
Cataract	1 (2%)	1 (3%)	–	1 (2%)	–
Phthisis bulbi	1 (2%)	1 (3%)	–	–	–
Bilateral, cataract	1 (2%)	–	–	–	–
Bilateral, retina, degeneration	8 (18%)	3 (8%)	5 (12%)	7 (16%)	5 (11%)
Cornea, inflammation, suppurative	–	–	–	1 (2%)	–
Cornea, necrosis	–	–	–	1 (2%)	–
Cornea, ulcer	–	–	–	1 (2%)	–
Retina, degeneration	4 (9%)	5 (13%)	2 (5%)	2 (5%)	4 (9%)
Sclera, metaplasia, osseous	2 (4%)	4 (11%)	2 (5%)	–	–
Harderian gland	(47)	(47)	(48)	(46)	(48)
Infiltration cellular, lymphocyte	1 (2%)	3 (6%)	–	–	–
Inflammation, chronic	1 (2%)	–	–	–	–
Inflammation, chronic active	–	–	1 (2%)	1 (2%)	–
Necrosis	–	–	–	–	1 (2%)
Acinus, degeneration	–	–	1 (2%)	1 (2%)	–
Epithelium, hyperplasia	1 (2%)	–	–	–	1 (2%)
Zymbal’s gland	(0)	(0)	(3)	(2)	(3)
Inflammation, suppurative	–	–	–	1 (50%)	–
Urinary System					
Kidney	(46)	(44)	(47)	(47)	(47)
Cyst	1 (2%)	–	–	1 (2%)	1 (2%)
Hyaline droplet	–	–	–	3 (6%)	1 (2%)
Hydronephrosis	1 (2%)	1 (2%)	–	–	2 (4%)
Necrosis	1 (2%)	–	–	–	–
Nephropathy	46 (100%)	40 (91%)	46 (98%)	45 (96%)	44 (94%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Pigmentation	–	–	1 (2%)	3 (6%)	2 (4%)
Transitional epithelium, hyperplasia	1 (2%)	–	1 (2%)	2 (4%)	–
Urethra	(0)	(0)	(0)	(0)	(1)
Urinary bladder	(48)	(43)	(48)	(47)	(47)
Hemorrhage	–	–	–	1 (2%)	–
Inflammation, chronic	1 (2%)	–	–	–	–
Lumen, dilatation	5 (10%)	5 (12%)	2 (4%)	5 (11%)	5 (11%)
Transitional epithelium, hyperplasia	–	–	1 (2%)	1 (2%)	2 (4%)

[a]Number of animals examined microscopically at the site and the number of animals with lesion.

Appendix B. Summary of Lesions in Female Rats in the Two-year Drinking Water Study of Glycidamide

Tables

Table B-1. Summary of the Incidence of Neoplasms in Female Rats in the Two-year Drinking Water Study of Glycidamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Disposition Summary					
Animals initially in study	48	48	48	48	48
Early deaths					
Moribund sacrifice	7	15	11	20	40
Natural deaths	2	–	4	3	3
Survivors					
Moribund sacrifice	3	6	5	8	3
Natural deaths	1	1	1	–	–
Terminal sacrifice	35	26	27	17	2
Animals examined microscopically	48	48	48	48	48
Alimentary System					
Esophagus	(48)	(48)	(48)	(48)	(48)
Intestine large, cecum	(46)	(48)	(44)	(45)	(45)
Adenoma	1 (2%)	–	–	–	–
Leukemia mononuclear	–	1 (2%)	–	1 (2%)	–
Lymphoma malignant	–	–	–	1 (2%)	–
Intestine large, colon	(46)	(48)	(44)	(45)	(45)
Adenoma	–	–	1 (2%)	–	–
Intestine large, rectum	(46)	(48)	(45)	(45)	(45)
Sarcoma, metastatic, clitoral gland	–	–	–	1 (2%)	–
Schwannoma malignant	–	–	1 (2%)	–	–
Intestine small, duodenum	(46)	(48)	(45)	(45)	(45)
Adenocarcinoma	–	1 (2%)	–	–	–
Leiomyoma	–	1 (2%)	1 (2%)	–	–
Intestine small, ileum	(46)	(48)	(44)	(45)	(45)
Leukemia mononuclear	–	–	–	–	2 (4%)
Intestine small, jejunum	(46)	(48)	(45)	(44)	(44)
Sarcoma, metastatic, uterus	–	1 (2%)	–	–	–
Liver	(48)	(48)	(48)	(48)	(48)
Hepatocellular adenoma, multiple	1 (2%)	–	–	–	–
Leukemia mononuclear	14 (29%)	11 (23%)	21 (44%)	19 (40%)	27 (56%)
Mesentery	(4)	(4)	(1)	(9)	(5)
Leukemia mononuclear	–	–	–	1 (11%)	1 (20%)
Lymphoma malignant	–	–	–	1 (11%)	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Oral mucosa	(1)	(2)	(5)	(4)	(9)
Squamous cell carcinoma	–	–	–	1 (25%)	2 (22%)
Squamous cell papilloma	1 (100%)	1 (50%)	2 (40%)	–	4 (44%)
Pancreas	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	2 (4%)	2 (4%)	4 (8%)	5 (10%)	4 (8%)
Lymphoma malignant	–	–	–	1 (2%)	–
Sarcoma, metastatic, uterus	–	1 (2%)	–	–	–
Salivary glands	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	2 (4%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Lymphoma malignant	–	–	–	1 (2%)	–
Stomach, forestomach	(48)	(48)	(48)	(47)	(46)
Lymphoma malignant	–	–	–	1 (2%)	–
Squamous cell papilloma	–	1 (2%)	–	–	3 (7%)
Stomach, glandular	(47)	(48)	(45)	(46)	(46)
Adenoma	–	–	–	–	1 (2%)
Leukemia mononuclear	1 (2%)	–	1 (2%)	1 (2%)	–
Lymphoma malignant	–	–	–	1 (2%)	–
Tongue	(0)	(1)	(1)	(3)	(4)
Squamous cell carcinoma	–	–	–	–	1 (25%)
Squamous cell papilloma	–	1 (100%)	–	1 (33%)	–
Cardiovascular System					
Blood Vessel	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	1 (2%)	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Heart	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	5 (10%)	4 (8%)	8 (17%)	8 (17%)	10 (21%)
Schwannoma malignant	2 (4%)	1 (2%)	4 (8%)	2 (4%)	1 (2%)
Endocrine System					
Adrenal cortex	(48)	(48)	(48)	(48)	(48)
Adenoma	–	–	1 (2%)	–	–
Carcinoma	1 (2%)	–	–	–	–
Leukemia mononuclear	2 (4%)	2 (4%)	4 (8%)	2 (4%)	5 (10%)
Lymphoma malignant	–	–	–	1 (2%)	–
Adrenal medulla	(47)	(48)	(47)	(47)	(48)
Leukemia mononuclear	2 (4%)	5 (10%)	5 (11%)	4 (9%)	7 (15%)
Pheochromocytoma benign	2 (4%)	1 (2%)	1 (2%)	4 (9%)	2 (4%)
Pheochromocytoma complex	–	–	–	–	1 (2%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Pheochromocytoma malignant	–	–	1 (2%)	1 (2%)	–
Bilateral, pheochromocytoma benign	1 (2%)	–	–	–	–
Islets, pancreatic	(48)	(48)	(48)	(48)	(47)
Adenoma	–	1 (2%)	–	–	–
Leukemia mononuclear	–	1 (2%)	–	1 (2%)	–
Lymphoma malignant	–	–	–	1 (2%)	–
Parathyroid gland	(48)	(45)	(48)	(47)	(46)
Pituitary gland	(47)	(48)	(47)	(46)	(48)
Leukemia mononuclear	1 (2%)	2 (4%)	2 (4%)	4 (9%)	5 (10%)
Pars distalis, adenoma	32 (68%)	36 (75%)	32 (68%)	27 (59%)	24 (50%)
Pars distalis, carcinoma	2 (4%)	2 (4%)	2 (4%)	–	–
Thyroid gland	(48)	(48)	(46)	(46)	(47)
Leukemia mononuclear	1 (2%)	–	–	2 (4%)	1 (2%)
C-cell, adenoma	1 (2%)	3 (6%)	6 (13%)	4 (9%)	2 (4%)
C-cell, adenoma, multiple	1 (2%)	1 (2%)	–	–	–
C-cell, carcinoma	1 (2%)	–	–	–	1 (2%)
Follicular cell, adenoma	–	3 (6%)	3 (7%)	1 (2%)	5 (11%)
Follicular cell, carcinoma	–	–	2 (4%)	3 (7%)	2 (4%)
Follicular cell, carcinoma, multiple	–	–	–	–	1 (2%)
General Body System					
Tissue NOS	(0)	(1)	(0)	(0)	(0)
Sarcoma, metastatic, uterus	–	1 (100%)	–	–	–
Genital System					
Clitoral gland	(48)	(48)	(48)	(48)	(47)
Adenoma	6 (13%)	3 (6%)	6 (13%)	3 (6%)	5 (11%)
Carcinoma	4 (8%)	6 (13%)	7 (15%)	11 (23%)	13 (28%)
Leukemia mononuclear	2 (4%)	2 (4%)	–	2 (4%)	2 (4%)
Sarcoma, deep invasion	–	–	–	1 (2%)	–
Squamous cell carcinoma	2 (4%)	–	–	–	–
Squamous cell papilloma	–	–	–	1 (2%)	2 (4%)
Bilateral, carcinoma	–	–	–	–	1 (2%)
Ovary	(48)	(48)	(48)	(48)	(48)
Granulosa cell tumor malignant	1 (2%)	–	–	–	–
Leukemia mononuclear	3 (6%)	1 (2%)	6 (13%)	3 (6%)	4 (8%)
Uterus	(48)	(48)	(48)	(48)	(48)
Adenoma	–	–	–	1 (2%)	1 (2%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Deciduoma benign	1 (2%)	–	–	–	–
Leukemia mononuclear	2 (4%)	–	1 (2%)	3 (6%)	2 (4%)
Lymphoma malignant	–	–	–	1 (2%)	–
Polyp stromal	9 (19%)	10 (21%)	12 (25%)	13 (27%)	10 (21%)
Polyp stromal, multiple	1 (2%)	1 (2%)	–	–	–
Sarcoma	–	1 (2%)	–	1 (2%)	–
Sarcoma stromal	1 (2%)	–	2 (4%)	1 (2%)	2 (4%)
Bilateral, polyp stromal	1 (2%)	–	1 (2%)	1 (2%)	1 (2%)
Vagina	(3)	(3)	(4)	(5)	(1)
Sarcoma, metastatic, clitoral gland	–	–	–	1 (20%)	–
Sarcoma stromal	–	–	–	–	1 (100%)
Hematopoietic System					
Bone marrow	(48)	(48)	(46)	(47)	(47)
Leukemia mononuclear	5 (10%)	7 (15%)	10 (22%)	11 (23%)	11 (23%)
Lymph node	(5)	(9)	(15)	(12)	(18)
Leukemia mononuclear	–	–	–	–	2 (11%)
Lymphoma malignant	–	–	–	1 (8%)	–
Axillary, leukemia mononuclear	1 (20%)	1 (11%)	1 (7%)	3 (25%)	–
Brachial, leukemia mononuclear	–	1 (11%)	–	–	–
Cervical, carcinoma, metastatic, thyroid gland	–	–	–	–	1 (6%)
Cervical, leukemia mononuclear	1 (20%)	1 (11%)	–	–	1 (6%)
Iliac, leukemia mononuclear	–	–	–	1 (8%)	–
Inguinal, leukemia mononuclear	–	1 (11%)	–	–	1 (6%)
Lumbar, leukemia mononuclear	2 (40%)	3 (33%)	2 (13%)	6 (50%)	3 (17%)
Mediastinal, leukemia mononuclear	2 (40%)	4 (44%)	5 (33%)	4 (33%)	3 (17%)
Mediastinal, lymphoma malignant	–	–	–	1 (8%)	–
Pancreatic, leukemia mononuclear	2 (40%)	5 (56%)	6 (40%)	5 (42%)	6 (33%)
Pancreatic, lymphoma malignant	–	–	–	1 (8%)	–
Renal, leukemia mononuclear	2 (40%)	2 (22%)	5 (33%)	3 (25%)	2 (11%)
Lymph node, mandibular	(48)	(48)	(47)	(47)	(47)
Leukemia mononuclear	5 (10%)	6 (13%)	10 (21%)	12 (26%)	15 (32%)
Lymphoma malignant	–	–	–	1 (2%)	–
Osteosarcoma, metastatic, nose	–	–	–	1 (2%)	–
Lymph node, mesenteric	(47)	(48)	(47)	(48)	(48)
Leukemia mononuclear	6 (13%)	7 (15%)	12 (26%)	14 (29%)	17 (35%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Lymphoma malignant	–	–	–	1 (2%)	–
Sarcoma, metastatic, uterus	–	1 (2%)	–	–	–
Spleen	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	14 (29%)	11 (23%)	21 (44%)	19 (40%)	27 (56%)
Lymphoma malignant	–	–	–	1 (2%)	–
Osteosarcoma, metastatic, nose	–	–	–	1 (2%)	–
Thymus	(45)	(47)	(45)	(45)	(44)
Leukemia mononuclear	2 (4%)	3 (6%)	3 (7%)	4 (9%)	6 (14%)
Lymphoma malignant	–	–	–	1 (2%)	–
Thymoma benign	–	–	1 (2%)	–	–
Integumentary System					
Mammary gland	(48)	(48)	(48)	(48)	(48)
Adenocarcinoma	1 (2%)	–	2 (4%)	2 (4%)	2 (4%)
Adenoma	2 (4%)	–	1 (2%)	–	3 (6%)
Adenoma, multiple	–	–	–	–	1 (2%)
Fibroadenoma	9 (19%)	15 (31%)	16 (33%)	10 (21%)	13 (27%)
Fibroadenoma, multiple	7 (15%)	11 (23%)	19 (40%)	23 (48%)	23 (48%)
Leukemia mononuclear	–	1 (2%)	–	–	–
Skin	(48)	(48)	(48)	(48)	(48)
Basal cell carcinoma	1 (2%)	–	–	–	–
Squamous cell carcinoma	–	–	–	–	1 (2%)
Subcutaneous tissue, fibroma	1 (2%)	–	–	1 (2%)	–
Subcutaneous tissue, lipoma	–	2 (4%)	1 (2%)	–	–
Musculoskeletal System					
Bone	(0)	(1)	(1)	(0)	(0)
Cranium, meningioma malignant, metastatic, brain, cerebrum	–	1 (100%)	–	–	–
Bone, femur	(48)	(48)	(48)	(48)	(48)
Chondrosarcoma	–	1 (2%)	–	–	–
Skeletal muscle	(48)	(48)	(48)	(48)	(48)
Rhabdomyosarcoma	–	1 (2%)	–	–	–
Sarcoma, metastatic, clitoral gland	–	–	–	1 (2%)	–
Nervous System					
Brain, brain stem	(48)	(48)	(47)	(48)	(48)
Carcinoma, metastatic, pituitary gland	2 (4%)	2 (4%)	2 (4%)	–	–
Leukemia mononuclear	–	1 (2%)	3 (6%)	3 (6%)	4 (8%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Brain, cerebellum	(48)	(48)	(47)	(48)	(48)
Astrocytoma malignant	–	–	–	1 (2%)	–
Granular cell tumor benign	–	–	–	1 (2%)	–
Histiocytic sarcoma	–	–	–	–	1 (2%)
Leukemia mononuclear	1 (2%)	–	3 (6%)	3 (6%)	2 (4%)
Meningioma malignant, metastatic, brain, cerebrum	–	1 (2%)	–	–	–
Brain, cerebrum	(48)	(48)	(48)	(48)	(48)
Astrocytoma malignant	–	–	–	–	1 (2%)
Carcinoma, metastatic, pituitary gland	–	–	1 (2%)	–	–
Glioma malignant	–	–	–	1 (2%)	1 (2%)
Leukemia mononuclear	–	–	5 (10%)	4 (8%)	3 (6%)
Meningioma malignant	–	1 (2%)	–	–	–
Peripheral nerve, sciatic	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	–	–	1 (2%)	1 (2%)	–
Spinal cord, cervical	(48)	(48)	(48)	(48)	(48)
Leukemia mononuclear	–	–	3 (6%)	2 (4%)	1 (2%)
Spinal cord, lumbar	(48)	(48)	(47)	(48)	(48)
Leukemia mononuclear	–	–	1 (2%)	2 (4%)	–
Spinal cord, thoracic	(48)	(48)	(47)	(48)	(48)
Leukemia mononuclear	–	1 (2%)	–	1 (2%)	1 (2%)
Respiratory System					
Lung	(48)	(48)	(48)	(48)	(48)
Alveolar/bronchiolar adenoma	–	–	1 (2%)	–	–
Carcinoma, metastatic, Zymbal's gland	1 (2%)	–	–	–	–
Leukemia mononuclear	5 (10%)	7 (15%)	11 (23%)	11 (23%)	15 (31%)
Lymphoma malignant	–	–	–	1 (2%)	–
Sarcoma, metastatic, uterus	–	1 (2%)	–	–	–
Nose	(48)	(48)	(48)	(48)	(47)
Adenoma	–	–	–	–	1 (2%)
Leukemia mononuclear	1 (2%)	–	–	–	–
Osteosarcoma	–	–	–	1 (2%)	–
Nasopharyngeal duct, squamous cell carcinoma	–	–	–	–	1 (2%)
Trachea	(48)	(48)	(47)	(47)	(48)
Special Senses System					
Eye	(46)	(47)	(45)	(45)	(45)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Leukemia mononuclear	1 (2%)	–	1 (2%)	–	–
Harderian gland	(48)	(48)	(46)	(47)	(47)
Leukemia mononuclear	1 (2%)	–	1 (2%)	–	–
Zymbal's gland	(1)	(0)	(0)	(0)	(2)
Carcinoma	1 (100%)	–	–	–	2 (100%)
Urinary System					
Kidney	(48)	(48)	(48)	(47)	(48)
Leukemia mononuclear	3 (6%)	2 (4%)	5 (10%)	4 (9%)	6 (13%)
Lymphoma malignant	–	–	–	1 (2%)	–
Urinary bladder	(48)	(48)	(48)	(47)	(47)
Leukemia mononuclear	–	1 (2%)	–	1 (2%)	1 (2%)
Sarcoma stromal, metastatic, uterus	–	–	1 (2%)	–	–
Transitional epithelium, papilloma	–	–	–	–	1 (2%)
Systemic Lesions					
Multiple organs	(48)[b]	(48)[b]	(48)[b]	(48)[b]	(48)[b]
Histiocytic sarcoma	–	–	–	–	1 (2%)
Leukemia mononuclear	14 (29%)	11 (23%)	21 (44%)	19 (40%)	27 (56%)
Lymphoma malignant	–	–	–	1 (2%)	–
Neoplasm Summary					
Total animals with primary neoplasms[c]	45	48	46	46	48
Total primary neoplasms	108	116	147	137	164
Total animals with benign neoplasms	40	47	42	40	44
Total benign neoplasms	77	91	105	91	102
Total animals with malignant neoplasms	22	20	31	34	44
Total malignant neoplasms	31	25	42	46	62
Total animals with metastatic neoplasms	2	4	3	2	1
Total metastatic neoplasms	3	9	4	5	1

[a]Number of animals examined microscopically at the site and the number of animals with neoplasm.
[b]Number of animals with any tissue examined microscopically.
[c]Primary neoplasms: all neoplasms except metastatic neoplasms.

Table B-2. Statistical Analysis of Neoplasms in Female Rats in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Adrenal Medulla: Benign Pheochromocytoma					
Overall rate[a]	3/47 (6%)	1/48 (2%)	1/47 (2%)	4/47 (9%)	2/48 (4%)
Adjusted rate[b]	7.2%	2.5%	2.6%	11.4%	8.0%
Terminal rate[c]	3/34 (9%)	1/26 (4%)	1/26 (4%)	2/17 (12%)	1/2 (50%)
First incidence (days)[d]	736 (T)	737 (T)	736 (T)	717	506
Poly-3 test[e]	P = 0.245	P = 0.320N	P = 0.330N	P = 0.405	P = 0.627
Brain (Cerebellum): Malignant Astrocytoma					
Overall rate	0/48 (0%)	0/48 (0%)	0/47 (0%)	1/48 (2%)	0/48 (0%)
Adjusted rate	0.0%	0.0%	0.0%	2.8%	0.0%
Terminal rate	0/35 (0%)	0/26 (0%)	0/27 (0%)	1/17 (6%)	0/2 (0%)
First incidence (days)	–	–	–	737 (T)	–
Poly-3 test	P = 0.363	–	–	P = 0.461	–
Brain (Cerebrum): Malignant Astrocytoma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	4.0%
Terminal rate	0/35 (0%)	0/26 (0%)	0/27 (0%)	0/17 (0%)	0/2 (0%)
First incidence (days)	–	–	–	–	520
Poly-3 test	P = 0.106	–	–	–	P = 0.397
Clitoral Gland: Adenoma					
Overall rate	6/48 (13%)	3/48 (6%)	6/48 (13%)	3/48 (6%)	5/47 (11%)
Adjusted rate	13.9%	7.5%	14.9%	8.4%	19.2%
Terminal rate	5/35 (14%)	3/26 (12%)	6/27 (22%)	1/17 (6%)	0/2 (0%)
First incidence (days)	665	736 (T)	736 (T)	697	465
Poly-3 test	P = 0.340	P = 0.278N	P = 0.570	P = 0.346N	P = 0.409
Clitoral Gland: Carcinoma					
Overall rate	4/48 (8%)	6/48 (13%)	7/48 (15%)	11/48 (23%)	14/47 (30%)
Adjusted rate	9.3%	14.5%	17.1%	30.0%	45.5%
Terminal rate	4/35 (11%)	3/26 (12%)	5/27 (19%)	6/17 (35%)	1/2 (50%)
First incidence (days)	736 (T)	570	513	480	374
Poly-3 test	P < 0.001***	P = 0.345	P = 0.233	P = 0.017*	P < 0.001***
Clitoral Gland: Squamous Cell Carcinoma					
Overall rate	2/48 (4%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/47 (0%)
Adjusted rate	4.7%	0.0%	0.0%	0.0%	0.0%
Terminal rate	2/35 (6%)	0/26 (0%)	0/27 (0%)	0/17 (0%)	0/2 (0%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
First incidence (days)	737 (T)	–	–	–	–
Poly-3 test	P = 0.111N	P = 0.252N	P = 0.252N	P = 0.283N	P = 0.387N
Clitoral Gland: Squamous Cell Papilloma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	2/47 (4%)
Adjusted rate	0.0%	0.0%	0.0%	2.8%	8.3%
Terminal rate	0/35 (0%)	0/26 (0%)	0/27 (0%)	1/17 (6%)	1/2 (50%)
First incidence (days)	–	–	–	736 (T)	620
Poly-3 test	P = 0.013*	–	–	P = 0.461	P = 0.136
Clitoral Gland: Adenoma, Carcinoma, and Squamous Cell Carcinoma and Papilloma (Combined)					
Overall rate	11/48 (23%)	9/48 (19%)	13/48 (27%)	14/48 (29%)	20/47 (43%)
Adjusted rate	25.5%	21.8%	31.7%	38.0%	60.3%
Terminal rate	10/35 (29%)	6/26 (23%)	11/27 (41%)	8/17 (47%)	2/2 (100%)
First incidence (days)	665	570	513	480	374
Poly-3 test	P < 0.001***	P = 0.442N	P = 0.347	P = 0.165	P = 0.001**
Heart: Malignant Schwannoma					
Overall rate	2/48 (4%)	1/48 (2%)	4/48 (8%)	2/48 (4%)	1/48 (2%)
Adjusted rate	4.6%	2.5%	9.8%	5.6%	4.1%
Terminal rate	1/35 (3%)	1/26 (4%)	2/27 (7%)	1/17 (6%)	0/2 (0%)
First incidence (days)	665	736 (T)	555	660	645
Poly-3 test	P = 0.509	P = 0.525N	P = 0.311	P = 0.622	P = 0.680N
Liver: Hepatocellular Adenoma					
Overall rate	1/48 (2%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)
Adjusted rate	2.3%	0.0%	0.0%	0.0%	0.0%
Terminal rate	1/35 (3%)	0/26 (0%)	0/27 (0%)	0/17 (0%)	0/2 (0%)
First incidence (days)	736 (T)	–	–	–	–
Poly-3 test	P = 0.265N	P = 0.513N	P = 0.513N	P = 0.539N	P = 0.606N
Mammary Gland: Adenoma					
Overall rate	2/48 (4%)	0/48 (0%)	1/48 (2%)	0/48 (0%)	4/48 (8%)
Adjusted rate	4.6%	0.0%	2.5%	0.0%	15.7%
Terminal rate	1/35 (3%)	0/26 (0%)	1/27 (4%)	0/17 (0%)	1/2 (50%)
First incidence (days)	714	–	737 (T)	–	550
Poly-3 test	P = 0.050	P = 0.252N	P = 0.524N	P = 0.284N	P = 0.144
Mammary Gland: Adenocarcinoma					
Overall rate	1/48 (2%)	0/48 (0%)	2/48 (4%)	2/48 (4%)	2/48 (4%)
Adjusted rate	2.3%	0.0%	4.9%	5.6%	8.0%
Terminal rate	0/35 (0%)	0/26 (0%)	1/27 (4%)	1/17 (6%)	0/2 (0%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
First incidence (days)	579	–	555	522	550
Poly-3 test	P = 0.102	P = 0.516N	P = 0.478	P = 0.435	P = 0.324
Mammary Gland: Fibroadenoma					
Overall rate	16/48 (33%)	26/48 (54%)	35/48 (73%)	33/48 (69%)	36/48 (75%)
Adjusted rate	35.9%	59.4%	81.4%	85.4%	90.7%
Terminal rate	10/35 (29%)	15/26 (58%)	22/27 (82%)	17/17 (100%)	2/2 (100%)
First incidence (days)	549	483	548	584	403
Poly-3 test	P < 0.001***	P = 0.019*	P < 0.001***	P < 0.001***	P < 0.001***
Mammary Gland: Adenocarcinoma and Fibroadenoma (Combined)					
Overall rate	17/48 (35%)	26/48 (54%)	36/48 (75%)	34/48 (71%)	36/48 (75%)
Adjusted rate	37.7%	59.4%	82.7%	86.5%	90.7%
Terminal rate	10/35 (29%)	15/26 (58%)	22/27 (82%)	17/17 (100%)	2/2 (100%)
First incidence (days)	549	483	548	522	403
Poly-3 test	P < 0.001***	P = 0.029*	P < 0.001***	P < 0.001***	P < 0.001***
Oral Mucosa: Squamous Cell Carcinoma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	2/48 (4%)
Adjusted rate	0.0%	0.0%	0.0%	2.8%	7.8%
Terminal rate	0/35 (0%)	0/26 (0%)	0/27 (0%)	1/17 (6%)	0/2 (0%)
First incidence (days)	–	–	–	737 (T)	421
Poly-3 test	P = 0.015*	–	–	P = 0.461	P = 0.145
Oral Mucosa: Squamous Cell Papilloma					
Overall rate	1/48 (2%)	1/48 (2%)	2/48 (4%)	0/48 (0%)	4/48 (8%)
Adjusted rate	2.3%	2.5%	5.0%	0.0%	15.3%
Terminal rate	1/35 (3%)	0/26 (0%)	2/27 (7%)	0/17 (0%)	0/2 (0%)
First incidence (days)	737 (T)	670	736 (T)	–	520
Poly-3 test	P = 0.045*	P = 0.747	P = 0.477	P = 0.539N	P = 0.070
Tongue: Squamous Cell Carcinoma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	4.0%
Terminal rate	0/35 (0%)	0/26 (0%)	0/27 (0%)	0/17 (0%)	0/2 (0%)
First incidence (days)	–	–	–	–	574
Poly-3 test	P = 0.105	–	–	–	P = 0.397
Tongue: Squamous Cell Papilloma					
Overall rate	0/48 (0%)	1/48 (2%)	0/48 (0%)	1/48 (2%)	0/48 (0%)
Adjusted rate	0.0%	2.5%	0.0%	2.8%	0.0%
Terminal rate	0/35 (0%)	0/26 (0%)	0/27 (0%)	0/17 (0%)	0/2 (0%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
First incidence (days)	–	693	–	584	–
Poly-3 test	P = 0.548	P = 0.488	–	P = 0.464	–
Oral Mucosa and Tongue (Combined): Squamous Cell Carcinoma and Papilloma (Combined)					
Overall rate	1/48 (2%)	2/48 (4%)	2/48 (4%)	2/48 (4%)	7/48 (15%)
Adjusted rate	2.3%	4.9%	5.0%	5.6%	25.0%
Terminal rate	1/35 (3%)	0/26 (0%)	2/27 (7%)	1/17 (6%)	0/2 (0%)
First incidence (days)	737 (T)	670	736 (T)	584	421
Poly-3 test	P = 0.001**	P = 0.481	P = 0.477	P = 0.437	P = 0.005**
Pituitary Gland (Pars Distalis): Adenoma					
Overall rate	32/47 (68%)	36/48 (75%)	32/47 (68%)	27/46 (59%)	24/48 (50%)
Adjusted rate	73.2%	80.5%	74.6%	68.7%	70.8%
Terminal rate	26/34 (77%)	20/26 (77%)	21/27 (78%)	11/17 (65%)	2/2 (100%)
First incidence (days)	572	519	513	435	374
Poly-3 test	P = 0.243N	P = 0.277	P = 0.540	P = 0.414N	P = 0.509N
Stomach (Forestomach): Squamous Cell Papilloma					
Overall rate	0/48 (0%)	1/48 (2%)	0/48 (0%)	0/47 (0%)	3/46 (7%)
Adjusted rate	0.0%	2.5%	0.0%	0.0%	12.4%
Terminal rate	0/35 (0%)	1/26 (4%)	0/27 (0%)	0/17 (0%)	0/2 (0%)
First incidence (days)	–	736 (T)	–	–	504
Poly-3 test	P = 0.015*	P = 0.487	–	–	P = 0.048*
Thyroid Gland: C-Cell Adenoma					
Overall rate	2/48 (4%)	4/48 (8%)	6/46 (13%)	4/46 (9%)	2/47 (4%)
Adjusted rate	4.7%	9.8%	15.3%	11.4%	8.1%
Terminal rate	2/35 (6%)	2/26 (8%)	5/27 (19%)	2/17 (12%)	0/2 (0%)
First incidence (days)	737 (T)	610	682	670	571
Poly-3 test	P = 0.358	P = 0.313	P = 0.104	P = 0.249	P = 0.485
Thyroid Gland: C-Cell Carcinoma					
Overall rate	1/48 (2%)	0/48 (0%)	0/46 (0%)	0/46 (0%)	1/47 (2%)
Adjusted rate	2.3%	0.0%	0.0%	0.0%	4.1%
Terminal rate	1/35 (3%)	0/26 (0%)	0/27 (0%)	0/17 (0%)	0/2 (0%)
First incidence (days)	736 (T)	–	–	–	705
Poly-3 test	P = 0.447	P = 0.513N	P = 0.519N	P = 0.542N	P = 0.618
Thyroid Gland: Follicular Cell Adenoma					
Overall rate	0/48 (0%)	3/48 (6%)	3/46 (7%)	1/46 (2%)	5/47 (11%)
Adjusted rate	0.0%	7.4%	7.6%	2.9%	19.3%
Terminal rate	0/35 (0%)	1/26 (4%)	2/27 (7%)	0/17 (0%)	1/2 (50%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
First incidence (days)	–	652	555	716	465
Poly-3 test	P = 0.017*	P = 0.109	P = 0.104	P = 0.459	P = 0.007**
Thyroid Gland: Follicular Cell Carcinoma					
Overall rate	0/48 (0%)	0/48 (0%)	2/46 (4%)	3/46 (7%)	3/47 (6%)
Adjusted rate	0.0%	0.0%	5.1%	8.6%	12.0%
Terminal rate	0/35 (0%)	0/26 (0%)	0/27 (0%)	3/17 (18%)	0/2 (0%)
First incidence (days)	–	–	634	737 (T)	596
Poly-3 test	P = 0.006**	–	P = 0.219	P = 0.085	P = 0.051
Thyroid Gland: Follicular Cell Adenoma and Carcinoma (Combined)					
Overall rate	0/48 (0%)	3/48 (6%)	5/46 (11%)	4/46 (9%)	8/47 (17%)
Adjusted rate	0.0%	7.4%	12.5%	11.5%	29.9%
Terminal rate	0/35 (0%)	1/26 (4%)	2/27 (7%)	3/17 (18%)	1/2 (50%)
First incidence (days)	–	652	555	716	465
Poly-3 test	P < 0.001***	P = 0.109	P = 0.025*	P = 0.037*	P < 0.001***
Uterus: Stromal Polyp					
Overall rate	11/48 (23%)	11/48 (23%)	13/48 (27%)	14/48 (29%)	11/48 (23%)
Adjusted rate	25.2%	26.3%	31.4%	38.1%	38.1%
Terminal rate	9/35 (26%)	7/26 (27%)	8/27 (30%)	8/17 (47%)	0/2 (0%)
First incidence (days)	572	570	513	584	453
Poly-3 test	P = 0.086	P = 0.550	P = 0.345	P = 0.155	P = 0.184
Uterus: Stromal Sarcoma					
Overall rate	1/48 (2%)	0/48 (0%)	2/48 (4%)	1/48 (2%)	2/48 (4%)
Adjusted rate	2.3%	0.0%	4.9%	2.8%	7.8%
Terminal rate	0/35 (0%)	0/26 (0%)	1/27 (4%)	0/17 (0%)	0/2 (0%)
First incidence (days)	721	–	619	364	453
Poly-3 test	P = 0.152	P = 0.514N	P = 0.480	P = 0.721	P = 0.332
Zymbal's Gland: Carcinoma					
Overall rate	1/48 (2%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	2/48 (4%)
Adjusted rate	2.3%	0.0%	0.0%	0.0%	7.8%
Terminal rate	0/35 (0%)	0/26 (0%)	0/27 (0%)	0/17 (0%)	0/2 (0%)
First incidence (days)	686	–	–	–	343
Poly-3 test	P = 0.134	P = 0.514N	P = 0.514N	P = 0.540N	P = 0.332
All Organs: Histiocytic Sarcoma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	4.1%
Terminal rate	0/35 (0%)	0/26 (0%)	0/27 (0%)	0/17 (0%)	0/2 (0%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
First incidence (days)	–	–	–	–	704
Poly-3 test	P = 0.105	–	–	–	P = 0.394
All Organs: Leukemia (Mononuclear)					
Overall rate	14/48 (29%)	11/48 (23%)	21/48 (44%)	19/48 (40%)	27/48 (56%)
Adjusted rate	30.6%	25.5%	47.4%	47.9%	72.6%
Terminal rate	8/35 (23%)	3/26 (12%)	10/27 (37%)	7/17 (42%)	1/2 (50%)
First incidence (days)	432	508	373	456	435
Poly-3 test	P < 0.001***	P = 0.382N	P = 0.076	P = 0.076	P < 0.001***
All Organs: Malignant Lymphoma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	0/48 (0%)
Adjusted rate	0.0%	0.0%	0.0%	2.8%	0.0%
Terminal rate	0/35 (0%)	0/26 (0%)	0/27 (0%)	0/17 (0%)	0/2 (0%)
First incidence (days)	–	–	–	435	–
Poly-3 test	P = 0.356	–	–	P = 0.466	–
All Organs: Osteosarcoma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	0/48 (0%)
Adjusted rate	0.0%	0.0%	0.0%	2.8%	0.0%
Terminal rate	0/35 (0%)	0/26 (0%)	0/27 (0%)	0/17 (0%)	0/2 (0%)
First incidence (days)	–	–	–	526	–
Poly-3 test	P = 0.357	–	–	P = 0.465	–
All Organs: Osteosarcoma or Osteoma					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	0/48 (0%)
Adjusted rate	0.0%	0.0%	0.0%	2.8%	0.0%
Terminal rate	0/35 (0%)	0/26 (0%)	0/27 (0%)	0/17 (0%)	0/2 (0%)
First incidence (days)	–	–	–	526	–
Poly-3 test	P = 0.357	–	–	P = 0.465	–
All Organs: Benign Neoplasms					
Overall rate	40/48 (83%)	47/48 (98%)	42/48 (88%)	40/48 (83%)	44/48 (92%)
Adjusted rate	87.9%	99.3%	93.6%	94.5%	97.9%
Terminal rate	32/35 (91%)	26/26 (100%)	26/27 (96%)	17/17 (100%)	2/2 (100%)
First incidence (days)	549	483	513	435	374
Poly-3 test	P = 0.089	P = 0.018*	P = 0.260	P = 0.201	P = 0.048*
All Organs: Malignant Neoplasms					
Overall rate	22/48 (46%)	20/48 (42%)	31/48 (65%)	34/48 (71%)	44/48 (92%)
Adjusted rate	47.5%	44.8%	67.1%	75.7%	96.4%
Terminal rate	14/35 (40%)	9/26 (35%)	16/27 (59%)	11/17 (65%)	2/2 (100%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
First incidence (days)	432	483	373	364	343
Poly-3 test	P < 0.001***	P = 0.481N	P = 0.042*	P = 0.004**	P < 0.001***
All Organs: Benign or Malignant Neoplasms					
Overall rate	45/48 (94%)	48/48 (100%)	46/48 (96%)	46/48 (96%)	48/48 (100%)
Adjusted rate	93.8%	100.0%	97.3%	99.2%	100.0%
Terminal rate	32/35 (92%)	26/26 (100%)	26/27 (96%)	17/17 (100%)	2/2 (100%)
First incidence (days)	432	483	373	364	343
Poly-3 test	P = 0.083	P = 0.119	P = 0.364	P = 0.180	P = 0.119

[a]Number of animals with neoplasm per number of animals examined microscopically.
[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c]Observed incidence at the terminal sacrifice.
[d]T indicates terminal sacrifice.
[e]Beneath the 0 mM Glycidamide are the p-values associated with the trend test. Beneath the treated (0.0875, 0.175, 0.35, and 0.70 mM Glycidamide) group incidences are the p-values corresponding to pairwise comparisons between the 0 mM Glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.

Table B-3. Historical Incidence of Thyroid Gland Follicular Cell Neoplasms in NCTR Control Female F344/N Nctr Rats

Study (Report Date)	Route of Administration	Incidence in Controls		
		Adenoma	Carcinoma	Adenoma or Carcinoma
Sulfamethazine (February 1988)	Diet	5/170 (2.9%)	0/170 (0.0%)	5/170 (2.9%)
Gentian Violet (November 1988)	Diet	1/159 (0.6%)	0/159 (0.0%)	1/159 (0.6%)
Doxylamine (April 1991)	Diet	0/47 (0.0%)	0/47 (0.0%)	0/47 (0.0%)
Triprolidine (June 1991)	Diet	1/45 (2.2%)	0/45 (0.0%)	1/45 (2.2%)
Pyrilamine (July 1991)	Diet	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Fumonisin B_1 (March 1999)	Diet	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Malachite Green (June 2001)	Diet	0/46 (0.0%)	0/46 (0.0%)	0/46 (0.0%)
Leucomalachite Green (June 2001)	Diet	0/46 (0.0%)	0/46 (0.0%)	0/46 (0.0%)
Acrylamide (February 2012)	Drinking Water	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Aloe vera Whole Leaf (2013)	Drinking Water	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Total (%) (All studies)		7/705 (0.1%)	0/705 (0.0%)	7/705 (0.1%)
Range		0.0–2.9%	0.0%	0.0–2.9%
Total (%) (Drinking water studies)		0/96 (0.0%)	0/96 (0.0%)	0/96 (0.0%)
Range		0.0%	0.0%	0.0%

Table B-4. Historical Incidence of Carcinoma of the Clitoral Gland in NCTR Control Female F344/N Nctr Rats

Study (Report Date)	Route of Administration	Incidence in Controls
Doxylamine (April 1991)	Diet	3/46 (6.5%)
Triprolidine (June 1991)	Diet	0/46 (0.0%)
Pyrilamine (July 1991)	Diet	3/45 (6.7%)
Fumonisin B_1 (March 1999)	Diet	1/41 (2.4%)
Malachite Green (June 2001)	Diet	5/48 (10.4%)
Leucomalachite Green (June 2001)	Diet	2/47 (4.3%)
Acrylamide (February 2012)	Drinking Water	1/48 (2.1%)
Aloe vera Whole Leaf (2013)	Drinking Water	3/48 (6.3%)
Total (%) (All studies)		18/369 (4.9%)
Range		0.0–10.4%
Total (%) (Drinking water studies)		4/96 (4.2%)
Range		2.1–6.3%

Table B-5. Historical Incidence of Fibroadenoma of the Mammary Gland in NCTR Control Female F344/N Nctr Rats

Study (Report Date)	Route of Administration	Incidence in Controls
Sulfamethazine (February 1988)	Diet	48/177 (27.1%)
Gentian Violet (November 1988)	Diet	65/169 (38.5%)
Doxylamine (April 1991)	Diet	19/48 (39.6%)
Triprolidine (June 1991)	Diet	15/46 (32.6%)
Pyrilamine (July 1991)	Diet	20/47 (42.6%)
Fumonisin B_1 (March 1999)	Diet	18/47 (38.3%)
Malachite Green (June 2001)	Diet	15/46 (32.6%)
Leucomalachite Green (June 2001)	Diet	20/48 (41.7%)
Acrylamide (February 2012)	Drinking Water	16/48 (33.3%)
Aloe vera Whole Leaf (2013)	Drinking Water	12/47 (25.5%)
Total (%) (All studies)		248/723 (34.3%)
Range		25.5–42.6%
Total (%) (Drinking water studies)		28/95 (29.5%)
Range		25.5–33.3%

Table B-6. Historical Incidence of Neoplasms of the Oral Cavity in NCTR Control Female F344/N Nctr Rats

Study (Report Date)	Route of Administration	Incidence in Controls		
		Papilloma or Squamous Cell Papilloma	Squamous Cell Carcinoma	Papilloma, Squamous Cell Papilloma, or Squamous Cell Carcinoma
Sulfamethazine (February 1988)	Diet	0/179 (0.0%)	0/179 (0.0%)	0/179 (0.0%)
Gentian Violet (November 1988)	Diet	1/167 (0.6%)	0/167 (0.0%)	1/167 (0.6%)
Doxylamine (April 1991)	Diet	–[a]	–[a]	–[a]
Triprolidine (June 1991)	Diet	–	–	–
Pyrilamine (July 1991)	Diet	1/48 (2.1%)	0/48 (0.0%)	1/48 (2.1%)
Fumonisin B_1 (March 1999)	Diet	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Malachite Green (June 2001)	Diet	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Leucomalachite Green (June 2001)	Diet	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Acrylamide (February 2012)	Drinking Water	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Aloe vera Whole Leaf (2013)	Drinking Water	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Total (%) (All studies)		2/634 (0.3%)	0/634 (0.0%)	2/634 (0.3%)
Range		0.0–2.1%	0.0%	0.0–2.1%
Total (%) (Drinking water studies)		0/96 (0.0%)	0/96 (0.0%)	0/96 (0.0%)
Range		0.0%	0.0%	0.0%

[a]Not reported.

Table B-7. Historical Incidence of Squamous Cell Papilloma or Carcinoma (Combined) of the Forestomach in NCTR Control Female F344/N Nctr Rats

Study (Report Date)	Route of Administration	Incidence in Controls
Sulfamethazine (February 1988)	Diet	0/179 (0.0%)
Gentian Violet (November 1988)	Diet	0/165 (0.0%)
Doxylamine (April 1991)	Diet	0/47 (0.0%)
Triprolidine (June 1991)	Diet	0/48 (0.0%)
Pyrilamine (July 1991)	Diet	0/48 (0.0%)
Fumonisin B_1 (March 1999)	Diet	0/48 (0.0%)
Malachite Green (June 2001)	Diet	0/48 (0.0%)
Leucomalachite Green (June 2001)	Diet	0/48 (0.0%)
Acrylamide (February 2012)	Drinking Water	0/48 (0.0%)
Aloe vera Whole Leaf (2013)	Drinking Water	1/48 (2.1%)
Total (%) (All studies)		1/727 (0.1%)
Range		0.0–2.1%
Total (%) (Drinking water studies)		1/96 (1.0%)
Range		0.0–2.1%

Table B-8. Historical Incidence of Mononuclear Cell Leukemia in NCTR Control Female F344/N Nctr Rats

Study (Report Date)	Route of Administration	Incidence in Controls
Sulfamethazine (February 1988)	Diet	67/179 (37.4%)
Gentian Violet (November 1988)	Diet	77/171 (45.0%)
Doxylamine (April 1991)	Diet	13/48 (27.1%)
Triprolidine (June 1991)	Diet	12/48 (25.0%)
Pyrilamine (July 1991)	Diet	6/48 (12.5%)
Fumonisin B_1 (March 1999)	Diet	13/48 (27.1%)
Malachite Green (June 2001)	Diet	19/48 (39.6%)
Leucomalachite Green (June 2001)	Diet	17/48 (35.4%)
Acrylamide (February 2012)	Drinking Water	10/48 (20.8%)
Aloe vera Whole Leaf (2013)	Drinking Water	10/48 (20.8%)
Total (%) (All studies)		244/734 (33.2%)
Range		12.5–45.0%
Total (%) (Drinking water studies)		20/96 (20.8%)
Range		20.8%

Table B-9. Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Two-year Drinking Water Study of Glycidamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Disposition Summary					
Animals initially in study	48	48	48	48	48
Early deaths					
Moribund sacrifice	7	15	11	20	40
Natural death	2	–	4	3	3
Survivors					
Moribund sacrifice	3	6	5	8	3
Natural death	1	1	1	–	–
Terminal sacrifice	35	26	27	17	2
Animals examined microscopically	48	48	48	48	48
Alimentary System					
Esophagus	(48)	(48)	(48)	(48)	(48)
Lumen, dilatation	–	–	–	1 (2%)	–
Intestine large, cecum	(46)	(48)	(44)	(45)	(45)
Ulcer	1 (2%)	–	–	–	–
Lumen, dilatation	–	1 (2%)	–	–	1 (2%)
Intestine large, colon	(46)	(48)	(44)	(45)	(45)
Goblet cell, hyperplasia	1 (2%)	–	–	–	–
Intestine large, rectum	(46)	(48)	(45)	(45)	(45)
Intestine small, duodenum	(46)	(48)	(45)	(45)	(45)
Intestine small, ileum	(46)	(48)	(44)	(45)	(45)
Infiltration cellular, lymphocyte	1 (2%)	–	–	–	–
Pigmentation	1 (2%)	–	–	–	–
Intestine small, jejunum	(46)	(48)	(45)	(44)	(44)
Infiltration cellular, lymphocyte	–	–	–	–	1 (2%)
Liver	(48)	(48)	(48)	(48)	(48)
Angiectasis	–	–	1 (2%)	–	1 (2%)
Basophilic focus	–	2 (4%)	2 (4%)	2 (4%)	4 (8%)
Basophilic focus, multiple	41 (85%)	38 (79%)	36 (75%)	35 (73%)	32 (67%)
Clear cell focus	2 (4%)	–	–	1 (2%)	–
Congestion	1 (2%)	1 (2%)	–	–	–
Deformity	7 (15%)	4 (8%)	4 (8%)	5 (10%)	7 (15%)
Degeneration, cystic	3 (6%)	2 (4%)	3 (6%)	2 (4%)	–
Eosinophilic focus	5 (10%)	8 (17%)	5 (10%)	4 (8%)	4 (8%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Eosinophilic focus, multiple	2 (4%)	3 (6%)	–	4 (8%)	–
Granuloma	3 (6%)	3 (6%)	–	1 (2%)	–
Hematopoietic cell proliferation	1 (2%)	–	1 (2%)	1 (2%)	2 (4%)
Hemorrhage	–	–	–	–	1 (2%)
Hepatodiaphragmatic nodule	2 (4%)	–	–	2 (4%)	1 (2%)
Hypertrophy	–	–	1 (2%)	–	–
Infiltration cellular, lymphocyte	–	1 (2%)	–	2 (4%)	–
Inflammation, chronic active	19 (40%)	14 (29%)	18 (38%)	13 (27%)	8 (17%)
Mitotic alteration	–	1 (2%)	–	–	–
Mixed cell focus	10 (21%)	7 (15%)	6 (13%)	3 (6%)	2 (4%)
Mixed cell focus, multiple	8 (17%)	8 (17%)	6 (13%)	5 (10%)	3 (6%)
Necrosis	2 (4%)	8 (17%)	1 (2%)	–	3 (6%)
Pigmentation	2 (4%)	4 (8%)	1 (2%)	–	1 (2%)
Thrombosis	–	–	–	2 (4%)	1 (2%)
Vacuolization cytoplasmic	15 (31%)	11 (23%)	18 (38%)	12 (25%)	7 (15%)
Bile duct, hyperplasia	16 (33%)	19 (40%)	25 (52%)	15 (31%)	15 (31%)
Hepatocyte, degeneration	4 (8%)	6 (13%)	3 (6%)	3 (6%)	4 (8%)
Hepatocyte, hyperplasia	–	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Mesentery	(4)	(4)	(1)	(9)	(5)
Cyst	–	–	–	1 (11%)	–
Infiltration cellular, lymphocyte	–	–	–	1 (11%)	–
Fat, necrosis	4 (100%)	4 (100%)	1 (100%)	7 (78%)	5 (100%)
Oral Mucosa	(1)	(2)	(5)	(4)	(9)
Epithelium, hyperplasia	–	1 (50%)	3 (60%)	3 (75%)	3 (33%)
Pancreas	(48)	(48)	(48)	(48)	(48)
Cyst	–	–	–	–	1 (2%)
Necrosis	–	–	–	2 (4%)	–
Acinus, degeneration	21 (44%)	18 (38%)	17 (35%)	19 (40%)	13 (27%)
Salivary glands	(48)	(48)	(48)	(48)	(48)
Infiltration cellular, lymphocyte	1 (2%)	–	–	–	–
Stomach, forestomach	(48)	(48)	(48)	(47)	(46)
Edema	–	–	–	–	1 (2%)
Fibrosis	1 (2%)	–	–	–	–
Inflammation, chronic active	1 (2%)	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Necrosis	–	–	1 (2%)	–	1 (2%)
Ulcer	–	2 (4%)	1 (2%)	2 (4%)	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Epithelium, hyperplasia	3 (6%)	3 (6%)	2 (4%)	2 (4%)	6 (13%)
Stomach, glandular	(47)	(48)	(45)	(46)	(46)
Necrosis	1 (2%)	1 (2%)	–	–	1 (2%)
Pigmentation	1 (2%)	–	–	–	–
Epithelium, degeneration	–	–	–	–	1 (2%)
Tongue	(0)	(1)	(1)	(3)	(4)
Inflammation, suppurative	–	–	–	1 (33%)	–
Keratin cyst	–	–	–	1 (33%)	–
Epithelium, hyperplasia	–	–	–	1 (33%)	1 (25%)
Cardiovascular System					
Blood vessel	(48)	(48)	(48)	(48)	(48)
Heart	(48)	(48)	(48)	(48)	(48)
Cardiomyopathy	40 (83%)	39 (81%)	38 (79%)	33 (69%)	31 (65%)
Thrombosis	–	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Endocardium, hyperplasia	–	1 (2%)	–	–	–
Ventricle, dilatation	–	1 (2%)	–	–	1 (2%)
Endocrine System					
Adrenal cortex	(48)	(48)	(48)	(48)	(48)
Accessory adrenal cortical nodule	–	–	–	3 (6%)	1 (2%)
Angiectasis	2 (4%)	3 (6%)	2 (4%)	2 (4%)	–
Congestion	1 (2%)	–	–	–	–
Cyst	–	–	1 (2%)	–	–
Degeneration, cystic	3 (6%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Hemorrhage	1 (2%)	–	–	–	–
Hyperplasia	1 (2%)	1 (2%)	–	2 (4%)	2 (4%)
Hypertrophy	4 (8%)	–	4 (8%)	–	–
Pigmentation	–	–	1 (2%)	–	–
Vacuolization cytoplasmic	12 (25%)	13 (27%)	12 (25%)	17 (35%)	22 (46%)
Adrenal medulla	(47)	(48)	(47)	(47)	(48)
Hyperplasia	3 (6%)	1 (2%)	4 (9%)	2 (4%)	–
Necrosis	–	–	–	–	1 (2%)
Islets, pancreatic	(48)	(48)	(48)	(48)	(47)
Hyperplasia	–	1 (2%)	–	2 (4%)	–
Parathyroid gland	(48)	(45)	(48)	(47)	(46)
Hyperplasia	2 (4%)	–	2 (4%)	–	3 (7%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Pituitary gland	(47)	(48)	(47)	(46)	(48)
Pars distalis, angiectasis	–	–	–	–	1 (2%)
Pars distalis, cyst	3 (6%)	3 (6%)	1 (2%)	1 (2%)	5 (10%)
Pars distalis, hyperplasia	11 (23%)	7 (15%)	12 (26%)	14 (30%)	20 (42%)
Pars intermedia, cyst	–	–	–	1 (2%)	
Pars intermedia, hyperplasia	–	–	–	1 (2%)	1 (2%)
Pars intermedia, Rathke's Cleft, Degeneration	–	–	–	1 (2%)	–
Thyroid gland	(48)	(48)	(46)	(46)	(47)
Cyst	–	–	–	1 (2%)	–
Infiltration cellular, polymorphonuclear	1 (2%)	–	–	–	–
C-cell, hyperplasia	31 (65%)	27 (56%)	24 (52%)	16 (35%)	19 (40%)
Follicular cell, hyperplasia	1 (2%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)
General Body System					
Tissue NOS	(0)	(1)	(0)	(0)	(0)
Genital System					
Clitoral gland	(48)	(48)	(48)	(48)	(47)
Fibrosis	–	–	–	1 (2%)	–
Hyperkeratosis	–	–	–	–	1 (2%)
Infiltration cellular, lymphocyte	1 (2%)	–	1 (2%)	1 (2%)	1 (2%)
Inflammation, suppurative	16 (33%)	13 (27%)	17 (35%)	20 (42%)	13 (28%)
Inflammation, chronic	–	1 (2%)	–	–	–
Inflammation, chronic active	5 (10%)	5 (10%)	8 (17%)	–	4 (9%)
Necrosis	–	2 (4%)	–	–	–
Acinus, degeneration	16 (33%)	21 (44%)	15 (31%)	16 (33%)	10 (21%)
Duct, ectasia	25 (52%)	21 (44%)	25 (52%)	17 (35%)	19 (40%)
Epithelium, hyperplasia	4 (8%)	2 (4%)	2 (4%)	1 (2%)	4 (9%)
Ovary	(48)	(48)	(48)	(48)	(48)
Atrophy	44 (92%)	46 (96%)	44 (92%)	46 (96%)	47 (98%)
Cyst	3 (6%)	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Bilateral, cyst	–	–	–	1 (2%)	–
Uterus	(48)	(48)	(48)	(48)	(48)
Angiectasis	–	–	1 (2%)	–	–
Cyst	2 (4%)	4 (8%)	5 (10%)	5 (10%)	4 (8%)
Decidual reaction	–	–	–	–	1 (2%)
Hemorrhage	1 (2%)	–	1 (2%)	–	1 (2%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Cervix, fibrosis	–	1 (2%)	–	3 (6%)	1 (2%)
Endometrium, hyperplasia, cystic	11 (23%)	17 (35%)	14 (29%)	14 (29%)	23 (48%)
Lumen, dilatation	6 (13%)	3 (6%)	6 (13%)	3 (6%)	3 (6%)
Vagina	(3)	(3)	(4)	(5)	(1)
Fibrosis	–	–	–	1 (20%)	–
Inflammation, suppurative	–	1 (33%)	–	–	–
Prolapse	–	–	1 (25%)	–	1 (100%)
Lumen, dilatation	3 (100%)	3 (100%)	3 (75%)	4 (80%)	–
Mucocyte, hyperplasia	2 (67%)	–	–	–	–
Hematopoietic System					
Bone marrow	(48)	(48)	(46)	(47)	(47)
Atrophy	–	–	–	1 (2%)	1 (2%)
Hyperplasia	2 (4%)	6 (13%)	7 (15%)	8 (17%)	14 (30%)
Lymph node	(5)	(9)	(15)	(12)	(18)
Lumbar, hyperplasia, lymphoid	–	1 (11%)	1 (7%)	1 (8%)	1 (6%)
Lumbar, infiltration cellular, plasma cell	–	–	1 (7%)	1 (8%)	1 (6%)
Lumbar, sinus, dilatation	–	–	–	1 (8%)	2 (11%)
Mediastinal, fibrosis	–	1 (11%)	–	–	–
Mediastinal, hemorrhage	–	1 (11%)	1 (7%)	–	–
Mediastinal, hyperplasia, lymphoid	–	–	1 (7%)	–	1 (6%)
Mediastinal, pigmentation	–	1 (11%)	1 (7%)	–	–
Mediastinal, sinus, dilatation	–	1 (11%)	–	–	–
Pancreatic, hyperplasia, lymphoid	1 (20%)	–	–	1 (8%)	1 (6%)
Pancreatic, infiltration cellular, histiocyte	–	–	1 (7%)	1 (8%)	–
Pancreatic, infiltration cellular, plasma cell	–	–	–	–	1 (6%)
Pancreatic, sinus, dilatation	–	–	2 (13%)	–	–
Renal, hemorrhage	1 (20%)	–	–	–	–
Renal, hyperplasia, lymphoid	–	–	–	–	2 (11%)
Renal, infiltration, cellular, histiocyte	–	–	1 (7%)	1 (8%)	1 (6%)
Renal, infiltration, cellular, plasma cell	–	–	–	–	1 (6%)
Renal, sinus, dilatation	1 (20%)	–	2 (13%)	–	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Sinus, thoracic, dilatation	–	–	–	1 (8%)	–
Thoracic, hemorrhage	–	–	–	1 (8%)	–
Thoracic, infiltration cellular, histiocyte	–	–	–	1 (8%)	–
Lymph node, mandibular	(48)	(48)	(47)	(47)	(47)
Hemorrhage	–	1 (2%)	1 (2%)	–	2 (4%)
Hyperplasia, lymphoid	6 (13%)	4 (8%)	7 (15%)	4 (9%)	9 (19%)
Infiltration cellular, plasma cell	22 (46%)	16 (33%)	18 (38%)	20 (43%)	16 (34%)
Sinus, dilatation	3 (6%)	5 (10%)	3 (6%)	5 (11%)	6 (13%)
Lymph node, mesenteric	(47)	(48)	(47)	(48)	(48)
Erythrophagocytosis	–	1 (2%)	–	–	–
Hemorrhage	1 (2%)	2 (4%)	–	4 (8%)	2 (4%)
Hyperplasia, lymphoid	10 (21%)	6 (13%)	6 (13%)	5 (10%)	10 (21%)
Infiltration cellular, histiocyte	–	–	–	–	1 (2%)
Infiltration cellular, mast cell	–	–	1 (2%)	1 (2%)	–
Infiltration cellular, plasma cell	2 (4%)	4 (8%)	6 (13%)	4 (8%)	6 (13%)
Sinus, dilatation	2 (4%)	2 (4%)	–	–	1 (2%)
Spleen	(48)	(48)	(48)	(48)	(48)
Accessory spleen	–	1 (2%)	–	1 (2%)	–
Congestion	–	–	1 (2%)	–	–
Depletion lymphoid	–	–	–	–	1 (2%)
Fibrosis	1 (2%)	3 (6%)	3 (6%)	5 (10%)	5 (10%)
Hematopoietic cell proliferation	14 (29%)	15 (31%)	9 (19%)	16 (33%)	11 (23%)
Hemorrhage	1 (2%)	–	1 (2%)	2 (4%)	–
Hyperplasia, lymphoid	1 (2%)	3 (6%)	–	2 (4%)	–
Hyperplasia, stromal	–	–	1 (2%)	–	–
Necrosis	1 (2%)	–	1 (2%)	1 (2%)	2 (4%)
Pigmentation	36 (75%)	34 (71%)	20 (42%)	21 (44%)	14 (29%)
Thymus	(45)	(47)	(45)	(45)	(44)
Atrophy	43 (96%)	44 (94%)	41 (91%)	40 (89%)	40 (91%)
Cyst	–	–	–	–	1 (2%)
Cyst, multilocular	–	–	1 (2%)	–	–
Integumentary System					
Mammary gland	(48)	(48)	(48)	(48)	(48)
Abscess	–	1 (2%)	–	–	–
Galactocele	7 (15%)	8 (17%)	12 (25%)	9 (19%)	3 (6%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Inflammation, chronic active	–	1 (2%)	–	–	–
Lactation	11 (23%)	10 (21%)	3 (6%)	2 (4%)	2 (4%)
Alveolus, hyperplasia	10 (21%)	6 (13%)	2 (4%)	1 (2%)	1 (2%)
Skin	(48)	(48)	(48)	(48)	(48)
Abscess	–	1 (2%)	–	–	–
Cyst epithelial inclusion	1 (2%)	–	2 (4%)	–	2 (4%)
Hemorrhage	–	–	–	1 (2%)	–
Infiltration cellular, plasma cell	–	1 (2%)	–	–	–
Inflammation, suppurative	3 (6%)	3 (6%)	–	3 (6%)	2 (4%)
Inflammation, chronic active	–	1 (2%)	–	2 (4%)	–
Ulcer	3 (6%)	2 (4%)	1 (2%)	4 (8%)	1 (2%)
Epithelium, hyperplasia	3 (6%)	2 (4%)	–	2 (4%)	1 (2%)
Musculoskeletal System					
Bone	(0)	(1)	(1)	(0)	(0)
Bone, femur	(48)	(48)	(48)	(48)	(48)
Fibrous osteodystrophy	–	3 (6%)	–	–	–
Osteopetrosis	–	–	–	3 (6%)	–
Skeletal muscle	(48)	(48)	(48)	(48)	(48)
Nervous System					
Brain, brain stem	(48)	(48)	(47)	(48)	(48)
Compression	10 (21%)	10 (21%)	12 (26%)	6 (13%)	11 (23%)
Hemorrhage	2 (4%)	1 (2%)	3 (6%)	–	–
Brain, cerebellum	(48)	(48)	(47)	(48)	(48)
Gliosis	–	–	1 (2%)	2 (4%)	1 (2%)
Hemorrhage	1 (2%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Ventricle, dilatation	1 (2%)	–	–	–	–
Brain, cerebrum	(48)	(48)	(48)	(48)	(48)
Cyst	–	–	–	1 (2%)	–
Gliosis	–	–	3 (6%)	2 (4%)	3 (6%)
Hemorrhage	1 (2%)	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Necrosis	–	–	–	–	1 (2%)
Ventricle, dilatation	4 (8%)	2 (4%)	2 (4%)	–	–
Ventricle, hemorrhage	–	–	1 (2%)	–	–
Peripheral nerve, sciatic	(48)	(48)	(48)	(48)	(48)
Axon, degeneration	27 (56%)	24 (50%)	32 (67%)	32 (67%)	20 (42%)
Spinal cord, cervical	(48)	(48)	(48)	(48)	(48)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Gliosis	–	–	–	1 (2%)	–
Hemorrhage	1 (2%)	–	–	–	–
Inflammation, chronic active	1 (2%)	–	–	–	–
Axon, degeneration	32 (67%)	19 (40%)	25 (52%)	14 (29%)	14 (29%)
Nerve, degeneration	2 (4%)	3 (6%)	3 (6%)	1 (2%)	–
Spinal cord, lumbar	(48)	(48)	(47)	(48)	(48)
Gliosis	–	–	2 (4%)	–	1 (2%)
Axon, degeneration	5 (10%)	6 (13%)	5 (11%)	6 (13%)	9 (19%)
Meninges, hyperplasia	–	–	–	1 (2%)	–
Meninges, infiltration cellular, histiocyte	–	–	1 (2%)	–	–
Nerve, degeneration	35 (73%)	35 (73%)	35 (74%)	30 (63%)	23 (48%)
Nerve, gliosis	1 (2%)	–	–	–	–
Nerve, hyperplasia	–	1 (2%)	–	–	–
Neuron, degeneration	–	–	–	–	1 (2%)
Spinal cord, thoracic	(48)	(48)	(47)	(48)	(48)
Hemorrhage	–	–	1 (2%)	–	–
Mineralization	–	1 (2%)	–	–	–
Axon, degeneration	29 (60%)	26 (54%)	18 (38%)	17 (35%)	18 (38%)
Meninges, hyperplasia	–	–	1 (2%)	1 (2%)	–
Meninges, infiltration cellular, histiocyte	–	–	–	1 (2%)	–
Nerve, degeneration	8 (17%)	7 (15%)	8 (17%)	8 (17%)	2 (4%)
Respiratory System					
Lung	(48)	(48)	(48)	(48)	(48)
Congestion	–	–	–	–	2 (4%)
Fibrosis	–	1 (2%)	–	–	–
Hemorrhage	1 (2%)	–	–	–	–
Infiltration cellular, histiocyte	24 (50%)	25 (52%)	24 (50%)	20 (42%)	9 (19%)
Inflammation, chronic active	–	–	1 (2%)	–	–
Necrosis	–	–	–	–	1 (2%)
Pigmentation	–	1 (2%)	–	–	–
Polyarteritis	–	–	–	–	1 (2%)
Alveolar epithelium, hyperplasia	3 (6%)	1 (2%)	3 (6%)	1 (2%)	4 (8%)
Nose	(48)	(48)	(48)	(48)	(47)
Foreign body	–	–	–	1 (2%)	–
Hyaline droplet	5 (10%)	6 (13%)	2 (4%)	5 (10%)	2 (4%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Inflammation, suppurative	1 (2%)	1 (2%)	2 (4%)	4 (8%)	1 (2%)
Inflammation, chronic active	2 (4%)	–	–	1 (2%)	1 (2%)
Goblet cell, hyperplasia	1 (2%)	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Trachea	(48)	(48)	(47)	(47)	(48)
Inflammation, suppurative	1 (2%)	–	–	–	–
Necrosis	1 (2%)	–	–	–	–
Ulcer	1 (2%)	–	–	–	–
Special Senses System					
Eye	(46)	(47)	(45)	(45)	(45)
Cataract	–	–	–	1 (2%)	2 (4%)
Inflammation, suppurative	1 (2%)	–	–	–	–
Bilateral, cataract	–	–	1 (2%)	–	–
Bilateral, retina, degeneration	5 (11%)	4 (9%)	3 (7%)	2 (4%)	2 (4%)
Retina, degeneration	7 (15%)	7 (15%)	6 (13%)	6 (13%)	4 (9%)
Harderian gland	(48)	(48)	(46)	(47)	(47)
Infiltration cellular, lymphocyte	8 (17%)	5 (10%)	1 (2%)	2 (4%)	8 (17%)
Inflammation, chronic	1 (2%)	–	–	–	–
Inflammation, chronic active	–	–	–	1 (2%)	–
Pigmentation	–	–	1 (2%)	–	–
Acinus, degeneration	–	–	1 (2%)	–	–
Epithelium, hyperplasia	–	–	–	1 (2%)	–
Zymbal’s gland	(1)	(0)	(0)	(0)	(2)
Urinary System					
Kidney	(48)	(48)	(48)	(47)	(48)
Cyst	–	–	–	1 (2%)	–
Hyaline droplet	1 (2%)	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Infarct	–	–	–	1 (2%)	–
Mineralization	34 (71%)	33 (69%)	29 (60%)	36 (77%)	30 (63%)
Nephropathy	43 (90%)	39 (81%)	38 (79%)	32 (68%)	25 (52%)
Pigmentation	–	–	–	1 (2%)	3 (6%)
Urinary Bladder	(48)	(48)	(48)	(47)	(47)
Infiltration cellular, histiocyte	–	–	–	–	1 (2%)
Lumen, dilatation	–	–	2 (4%)	–	1 (2%)

[a]Number of animals examined microscopically at the site and the number of animals with lesion.

Appendix C. Summary of Lesions in Male Mice in the Two-year Drinking Water Study of Glycidamide

Tables

Table C-1. Summary of the Incidence of Neoplasms in Male Mice in the Two-year Drinking Water Study of Glycidamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Disposition Summary					
Animals initially in study	48	48	48	48	48
Early deaths					
Moribund sacrifice	–	4	11	16	11
Natural deaths	1	3	2	3	9
Survivors					
Moribund sacrifice	2	–	1	2	2
Natural deaths	–	–	–	1	1
Terminal sacrifice	45	41	34	26	25
Animals examined microscopically	48	48	48	48	–
Alimentary System					
Esophagus	(47)	(48)	(47)	(47)	(46)
Gallbladder	(46)	(45)	(46)	(45)	(39)
Lymphoma malignant	–	1 (2%)	–	–	–
Intestine large, cecum	(47)	(45)	(46)	(44)	(39)
Histiocytic sarcoma	–	–	–	1 (2%)	–
Intestine large, colon	(47)	(45)	(47)	(45)	(39)
Intestine large, rectum	(47)	(45)	(47)	(45)	(39)
Intestine small, duodenum	(47)	(45)	(46)	(44)	(39)
Adenoma	–	–	–	1 (2%)	–
Lymphoma malignant	–	–	–	1 (2%)	1 (3%)
Intestine small, ileum	(47)	(45)	(46)	(44)	(39)
Lymphoma malignant	2 (4%)	–	–	–	1 (3%)
Intestine small, jejunum	(47)	(45)	(46)	(44)	(39)
Lymphoma malignant	1 (2%)	–	1 (2%)	1 (2%)	–
Liver	(47)	(47)	(47)	(47)	(48)
Hemangiosarcoma	–	–	1 (2%)	–	–
Hepatocellular adenoma	9 (19%)	1 (2%)	8 (17%)	8 (17%)	10 (21%)
Hepatocellular adenoma, multiple	1 (2%)	–	–	4 (9%)	1 (2%)
Hepatocellular carcinoma	5 (11%)	9 (19%)	6 (13%)	7 (15%)	9 (19%)
Hepatocellular carcinoma, multiple	–	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Hepatocholangiocarcinoma	–	–	–	1 (2%)	2 (4%)
Histiocytic sarcoma	1 (2%)	–	1 (2%)	3 (6%)	2 (4%)
Leukemia	–	–	–	–	1 (2%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Lymphoma malignant	–	1 (2%)	–	2 (4%)	3 (6%)
Mesentery	(2)	(1)	(0)	(1)	(1)
Fibrous histiocytoma	1 (50%)	–	–	–	–
Hepatocholangiocarcinoma, metastatic, liver	–	–	–	–	1 (100%)
Oral mucosa	(0)	(1)	(0)	(0)	(2)
Squamous cell carcinoma	–	–	–	–	1 (50%)
Squamous cell papilloma	–	–	–	–	1 (50%)
Pancreas	(47)	(46)	(47)	(47)	(45)
Fibrous histiocytoma	1 (2%)	–	–	–	–
Histiocytic sarcoma	1 (2%)	–	–	1 (2%)	–
Leukemia	–	–	–	–	1 (2%)
Lymphoma malignant	–	1 (2%)	–	1 (2%)	–
Salivary glands	(47)	(47)	(47)	(46)	(44)
Leukemia	–	–	–	–	1 (2%)
Lymphoma malignant	–	1 (2%)	–	–	–
Stomach, forestomach	(47)	(45)	(48)	(45)	(41)
Squamous cell carcinoma	–	–	–	–	2 (5%)
Squamous cell papilloma	–	2 (4%)	3 (6%)	1 (2%)	9 (22%)
Squamous cell papilloma, multiple	–	–	–	1 (2%)	1 (2%)
Stomach, glandular	(47)	(45)	(46)	(44)	(38)
Tongue	(0)	(0)	(0)	(1)	(0)
Squamous cell carcinoma	–	–	–	1 (100%)	–
Cardiovascular System					
Blood vessel	(47)	(48)	(47)	(47)	(47)
Hepatocholangiocarcinoma, metastatic, liver	–	–	–	–	1 (2%)
Leukemia	–	–	–	–	1 (2%)
Lymphoma malignant	–	–	–	–	3 (6%)
Heart	(47)	(48)	(47)	(47)	(46)
Hepatocholangiocarcinoma, metastatic, liver	–	–	–	–	1 (2%)
Histiocytic sarcoma	–	–	–	1 (2%)	–
Leukemia	–	–	–	–	1 (2%)
Lymphoma malignant	–	–	–	1 (2%)	2 (4%)
Endocrine System					
Adrenal cortex	(47)	(47)	(47)	(45)	(44)
Hepatocholangiocarcinoma, metastatic, liver	–	–	–	–	1 (2%)
Histiocytic sarcoma	–	–	–	1 (2%)	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Leukemia	–	–	–	–	1 (2%)
Lymphoma malignant	–	–	–	1 (2%)	2 (5%)
Adrenal medulla	(47)	(46)	(47)	(45)	(43)
Leukemia	–	–	–	–	1 (2%)
Pheochromocytoma benign	1 (2%)	1 (2%)	–	–	1 (2%)
Islets, pancreatic	(47)	(47)	(47)	(47)	(44)
Adenoma	–	–	–	–	1 (2%)
Histiocytic sarcoma	–	–	–	1 (2%)	–
Lymphoma malignant	–	1 (2%)	–	–	–
Parathyroid gland	(43)	(48)	(43)	(42)	(42)
Pituitary gland	(45)	(44)	(47)	(42)	(42)
Lymphoma malignant	–	–	–	1 (2%)	–
Pars distalis, carcinoma	–	–	–	1 (2%)	–
Thyroid gland	(47)	(48)	(47)	(44)	(43)
Lymphoma malignant	–	1 (2%)	–	–	–
Follicular cell, carcinoma	–	–	1 (2%)	–	–
General Body System					
Tissue NOS	(0)	(0)	(0)	(1)	(1)
Histiocytic sarcoma	–	–	–	1 (100%)	–
Thoracic, hepatocholangiocarcinoma, metastatic, liver	–	–	–	–	1 (100%)
Genital System					
Epididymis	(47)	(47)	(47)	(46)	(43)
Histiocytic sarcoma	–	–	–	1 (2%)	–
Leukemia	–	–	–	–	1 (2%)
Penis	(0)	(0)	(1)	(0)	(0)
Preputial gland	(47)	(47)	(46)	(46)	(44)
Hemangioma	–	–	1 (2%)	–	–
Leukemia	–	–	–	–	1 (2%)
Squamous cell carcinoma	–	–	–	1 (2%)	–
Prostate	(47)	(47)	(47)	(45)	(41)
Fibrous histiocytoma	1 (2%)	–	–	–	–
Histiocytic sarcoma	–	–	–	1 (2%)	–
Leukemia	–	–	–	–	1 (2%)
Lymphoma malignant	–	–	–	–	1 (2%)
Seminal vesicle	(47)	(46)	(47)	(45)	(41)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Histiocytic sarcoma	–	–	–	1 (2%)	–
Leukemia	–	–	–	–	1 (2%)
Testes	(47)	(46)	(46)	(46)	(42)
Histiocytic sarcoma	–	–	–	1 (2%)	–
Leukemia	–	–	–	–	1 (2%)
Hematopoietic System					
Bone marrow	(47)	(47)	(47)	(46)	(44)
Leukemia	–	–	–	–	1 (2%)
Lymphoma malignant	–	–	–	–	1 (2%)
Lymph node	(4)	(5)	(2)	(6)	(6)
Axillary, fibrous histiocytoma	1 (25%)	–	–	–	–
Axillary, lymphoma malignant	–	–	–	–	1 (17%)
Inguinal, fibrous histiocytoma	1 (25%)	–	–	–	–
Inguinal, lymphoma malignant	–	1 (20%)	–	–	1 (17%)
Lumbar, fibrous histiocytoma	1 (25%)	–	–	–	–
Lumbar, lymphoma malignant	1 (25%)	1 (20%)	–	1 (17%)	–
Mediastinal, fibrous histiocytoma	1 (25%)	–	–	–	–
Mediastinal, lymphoma malignant	1 (25%)	1 (20%)	–	1 (17%)	1 (17%)
Pancreatic, lymphoma malignant	–	–	–	1 (17%)	1 (17%)
Renal, histiocytic sarcoma	1 (25%)	–	–	1 (17%)	–
Renal, lymphoma malignant	1 (25%)	2 (40%)	–	1 (17%)	1 (17%)
Lymph node, mandibular	(47)	(47)	(47)	(45)	(43)
Fibrous histiocytoma	1 (2%)	–	–	–	–
Histiocytic sarcoma	1 (2%)	–	–	–	–
Leukemia	–	–	–	–	1 (2%)
Lymphoma malignant	1 (2%)	1 (2%)	–	1 (2%)	2 (5%)
Lymph node, mesenteric	(47)	(46)	(47)	(45)	(42)
Fibrous histiocytoma	1 (2%)	–	–	–	–
Histiocytic sarcoma	1 (2%)	–	1 (2%)	2 (4%)	1 (2%)
Leukemia	–	–	–	–	1 (2%)
Lymphoma malignant	2 (4%)	3 (7%)	2 (4%)	4 (9%)	6 (14%)
Spleen	(47)	(47)	(47)	(46)	(44)
Basosquamous tumor malignant, metastatic, skin	–	–	–	–	1 (2%)
Hemangiosarcoma	2 (4%)	–	1 (2%)	1 (2%)	2 (5%)
Histiocytic sarcoma	1 (2%)	–	1 (2%)	2 (4%)	1 (2%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Leukemia	–	–	–	–	1 (2%)
Lymphoma malignant	1 (2%)	4 (9%)	2 (4%)	4 (9%)	6 (14%)
Thymus	(43)	(45)	(43)	(39)	(34)
Histiocytic sarcoma	1 (2%)	–	–	–	–
Lymphoma malignant	1 (2%)	3 (7%)	–	1 (3%)	5 (15%)
Integumentary System					
Mammary gland	(3)	(1)	(0)	(2)	(1)
Skin	(47)	(48)	(47)	(47)	(46)
Basosquamous tumor malignant	–	–	–	–	2 (4%)
Fibroma	–	1 (2%)	–	–	–
Fibroma, multiple	–	–	1 (2%)	–	–
Keratoacanthoma	–	–	–	–	1 (2%)
Squamous cell carcinoma	–	–	–	–	2 (4%)
Squamous cell papilloma	–	1 (2%)	2 (4%)	1 (2%)	8 (17%)
Subcutaneous tissue, fibrosarcoma	2 (4%)	–	4 (9%)	2 (4%)	3 (7%)
Subcutaneous tissue, fibrous histiocytoma	1 (2%)	2 (4%)	–	–	1 (2%)
Subcutaneous tissue, hemangiosarcoma	–	1 (2%)	–	–	1 (2%)
Subcutaneous tissue, sarcoma	–	1 (2%)	–	2 (4%)	–
Subcutaneous tissue, schwannoma malignant	–	1 (2%)	4 (9%)	3 (6%)	–
Musculoskeletal System					
Bone, femur	(48)	(48)	(48)	(48)	(47)
Skeletal muscle	(48)	(47)	(47)	(47)	(47)
Hemangiosarcoma	1 (2%)	–	–	–	–
Hepatocholangiocarcinoma, metastatic, liver	–	–	–	–	1 (2%)
Histiocytic sarcoma	–	–	–	1 (2%)	–
Sarcoma	–	–	–	1 (2%)	–
Nervous System					
Brain, brain stem	(47)	(47)	(46)	(46)	(42)
Leukemia	–	–	–	–	1 (2%)
Brain, cerebellum	(47)	(47)	(46)	(46)	(42)
Leukemia	–	–	–	–	1 (2%)
Brain, cerebrum	(47)	(47)	(46)	(46)	(42)
Leukemia	–	–	–	–	1 (2%)
Peripheral nerve, sciatic	(47)	(47)	(47)	(46)	(42)
Schwannoma malignant, metastatic, skin	–	–	1 (2%)	–	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Spinal cord, cervical	(47)	(46)	(46)	(44)	(39)
Leukemia	–	–	–	–	1 (3%)
Spinal cord, lumbar	(47)	(46)	(46)	(45)	(39)
Leukemia	–	–	–	–	1 (3%)
Spinal cord, thoracic	(47)	(46)	(46)	(45)	(39)
Leukemia	–	–	–	–	1 (3%)
Respiratory System					
Lung	(47)	(46)	(47)	(47)	(47)
Adenocarcinoma, metastatic, harderian gland	–	–	–	–	1 (2%)
Alveolar/bronchiolar adenoma	–	6 (13%)	5 (11%)	8 (17%)	13 (28%)
Alveolar/bronchiolar adenoma, multiple	–	1 (2%)	2 (4%)	5 (11%)	4 (9%)
Alveolar/bronchiolar carcinoma	–	–	1 (2%)	–	2 (4%)
Basosquamous tumor malignant, metastatic, skin	–	–	–	–	1 (2%)
Fibrosarcoma, metastatic, skin	–	–	1 (2%)	–	–
Hepatocellular carcinoma, metastatic, liver	1 (2%)	–	1 (2%)	1 (2%)	–
Hepatocholangiocarcinoma, metastatic, liver	–	–	–	1 (2%)	1 (2%)
Histiocytic sarcoma	–	–	1 (2%)	3 (6%)	1 (2%)
Leukemia	–	–	–	–	1 (2%)
Lymphoma malignant	1 (2%)	2 (4%)	–	1 (2%)	3 (6%)
Sarcoma, metastatic, skeletal muscle	–	–	–	1 (2%)	–
Nose	(47)	(46)	(47)	(47)	(45)
Fibrous histiocytoma	1 (2%)	–	–	–	–
Hepatocholangiocarcinoma, metastatic, liver	–	–	–	–	1 (2%)
Leukemia	–	–	–	–	1 (2%)
Lymphoma malignant	–	–	–	–	1 (2%)
Trachea	(47)	(48)	(46)	(46)	(45)
Special Senses System					
Eye	(47)	(45)	(46)	(44)	(42)
Harderian gland	(47)	(47)	(47)	(46)	(47)
Adenocarcinoma	–	–	–	–	1 (2%)
Adenoma	3 (6%)	15 (32%)	19 (40%)	17 (37%)	11 (23%)
Leukemia	–	–	–	–	1 (2%)
Bilateral, adenoma	–	2 (4%)	4 (9%)	15 (33%)	31 (66%)
Zymbal’s gland	(0)	(0)	(0)	(0)	(1)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Urinary System					
Kidney	(47)	(47)	(47)	(46)	(46)
Hepatocholangiocarcinoma, metastatic, liver	–	–	–	–	1 (2%)
Histiocytic sarcoma	–	–	–	1 (2%)	–
Leukemia	–	–	–	–	1 (2%)
Lymphoma malignant	–	1 (2%)	–	1 (2%)	2 (4%)
Renal tubule, adenoma	–	–	–	–	1 (2%)
Renal tubule, carcinoma	–	–	–	–	2 (4%)
Urinary bladder	(47)	(46)	(47)	(45)	(41)
Hemangioma	–	–	–	–	1 (2%)
Leukemia	–	–	–	–	1 (2%)
Lymphoma malignant	–	–	–	–	1 (2%)
Transitional epithelium, papilloma	–	–	–	–	1 (2%)
Systemic Lesions					
Multiple organs	(48)[b]	(48)[b]	(48)[b]	(48)[b]	(48)[b]
Histiocytic sarcoma	1 (2%)	–	1 (2%)	3 (6%)	3 (6%)
Leukemia	–	–	–	–	1 (2%)
Lymphoma malignant	2 (4%)	4 (8%)	2 (4%)	4 (8%)	7 (15%)
Neoplasm Summary					
Total animals with primary neoplasms[c]	23	35	41	46	47
Total primary neoplasms	38	49	67	90	138
Total animals with benign neoplasms	14	23	34	40	43
Total benign neoplasms	14	30	45	61	95
Total animals with malignant neoplasms	13	17	21	27	33
Total malignant neoplasms	24	19	22	29	43
Total animals with metastatic neoplasms	1	–	3	3	3
Total metastatic neoplasms	1	–	3	3	12

[a]Number of animals examined microscopically at the site and the number of animals with neoplasm.
[b]Number of animals with any tissue examined microscopically.
[c]Primary neoplasms: all neoplasms except metastatic neoplasms.

Table C-2. Statistical Analysis of Neoplasms in Male Mice in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Harderian Gland: Adenoma					
Overall rate[a]	3/47 (6%)	17/47 (36%)	23/47 (49%)	32/46 (70%)	42/47 (89%)
Adjusted rate[b]	6.4%	38.3%	52.1%	77.3%	93.4%
Terminal rate[c]	3/45 (7%)	17/41 (42%)	16/34 (47%)	20/26 (77%)	25/25 (100%)
First incidence (days)[d]	733 (T)	726 (T)	558	559	530
Poly-3 test[e]	P < 0.001***	P < 0.001***	P < 0.001***	P < 0.001***	P < 0.001***
Harderian Gland: Adenocarcinoma					
Overall rate	0/47 (0%)	0/47 (0%)	0/47 (0%)	0/46 (0%)	1/47 (2%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	2.6%
Terminal rate	0/45 (0%)	0/41 (0%)	0/34 (0%)	0/26 (0%)	1/25 (4%)
First incidence (days)	–	–	–	–	733 (T)
Poly-3 test	P = 0.129	–	–	–	P = 0.460
Harderian Gland: Bilateral Adenoma					
Overall rate	0/47 (0%)	2/47 (4%)	4/47 (9%)	15/46 (33%)	31/47 (66%)
Adjusted rate	0.0%	4.5%	9.5%	38.2%	73.1%
Terminal rate	0/45 (0%)	2/41 (5%)	2/34 (6%)	9/26 (35%)	20/25 (80%)
First incidence (days)	–	733 (T)	618	646	530
Poly-3 test	P < 0.001***	P = 0.226	P = 0.048*	P < 0.001***	P < 0.001***
Harderian Gland: Adenoma and Adenocarcinoma (Combined)					
Overall rate	3/47 (6%)	17/47 (36%)	23/47 (49%)	32/46 (70%)	42/47 (89%)
Adjusted rate	6.4%	38.3%	52.1%	77.3%	93.4%
Terminal rate	3/45 (7%)	17/41 (42%)	16/34 (47%)	20/26 (77%)	25/25 (100%)
First incidence (days)	733 (T)	726 (T)	558	559	530
Poly-3 test	P < 0.001***	P < 0.001***	P < 0.001***	P < 0.001***	P < 0.001***
Lung: Alveolar/Bronchiolar Adenoma					
Overall rate	0/47 (0%)	7/46 (15%)	7/47 (15%)	13/47 (28%)	17/47 (36%)
Adjusted rate	0.0%	16.0%	16.6%	32.3%	42.1%
Terminal rate	0/45 (0%)	7/41 (17%)	6/34 (18%)	8/26 (31%)	10/25 (40%)
First incidence (days)	–	726 (T)	604	552	530
Poly-3 test	P < 0.001***	P = 0.006**	P = 0.005**	P < 0.001***	P < 0.001***
Lung: Alveolar/Bronchiolar Carcinoma					
Overall rate	0/47 (0%)	0/46 (0%)	1/47 (2%)	0/47 (0%)	2/47 (4%)
Adjusted rate	0.0%	0.0%	2.4%	0.0%	5.2%
Terminal rate	0/45 (0%)	0/41 (0%)	1/34 (3%)	0/26 (0%)	1/25 (4%)
First incidence (days)	–	–	733 (T)	–	669
Poly-3 test	P = 0.058	–	P = 0.477	–	P = 0.196

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Lung: Alveolar/Bronchiolar Adenoma and Carcinoma (Combined)					
Overall rate	0/47 (0%)	7/46 (15%)	8/47 (17%)	13/47 (28%)	19/47 (40%)
Adjusted rate	0.0%	16.0%	19.0%	32.3%	46.7%
Terminal rate	0/45 (0%)	7/41 (17%)	7/34 (21%)	8/26 (31%)	11/25 (44%)
First incidence (days)	–	726 (T)	604	552	530
Poly-3 test	P < 0.001***	P = 0.006**	P = 0.002**	P < 0.001***	P < 0.001***
Skin: Squamous Cell Carcinoma					
Overall rate	0/47 (0%)	0/48 (0%)	0/47 (0%)	0/47 (0%)	2/46 (4%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	5.4%
Terminal rate	0/45 (0%)	0/41 (0%)	0/34 (0%)	0/26 (0%)	2/25 (8%)
First incidence (days)	–	–	–	–	733 (T)
Poly-3 test	P = 0.017*	–	–	–	P = 0.191
Skin: Squamous Cell Papilloma					
Overall rate	0/47 (0%)	1/48 (2%)	2/47 (4%)	1/47 (2%)	8/46 (17%)
Adjusted rate	0.0%	2.2%	4.8%	2.6%	20.7%
Terminal rate	0/45 (0%)	1/41 (2%)	2/34 (6%)	1/26 (4%)	4/25 (16%)
First incidence (days)	–	733 (T)	733 (T)	733 (T)	574
Poly-3 test	P < 0.001***	P = 0.493	P = 0.213	P = 0.462	P = 0.001**
Skin: Squamous Cell Carcinoma and Papilloma (Combined)					
Overall rate[a]	0/47 (0%)	1/48 (2%)	2/47 (4%)	1/47 (2%)	9/46 (20%)
Adjusted rate[b]	0.0%	2.2%	4.8%	2.6%	23.3%
Terminal rate[c]	0/45 (0%)	1/41 (2%)	2/34 (6%)	1/26 (4%)	5/25 (20%)
First incidence (days)[d]	–	733 (T)	733 (T)	733 (T)	574
Poly-3 test[e]	P < 0.001***	P = 0.493	P = 0.213	P = 0.462	P < 0.001***
Stomach (Forestomach): Squamous Cell Carcinoma					
Overall rate	0/47 (0%)	0/45 (0%)	0/48 (0%)	0/45 (0%)	2/41 (5%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	5.8%
Terminal rate	0/45 (0%)	0/41 (0%)	0/34 (0%)	0/26 (0%)	2/25 (8%)
First incidence (days)	–	–	–	–	726 (T)
Poly-3 test	P = 0.014*	–	–	–	P = 0.176
Stomach (Forestomach): Squamous Cell Papilloma					
Overall rate	0/47 (0%)	2/45 (4%)	3/48 (6%)	2/45 (4%)	10/41 (24%)
Adjusted rate	0.0%	4.7%	7.2%	5.3%	27.6%
Terminal rate	0/45 (0%)	2/41 (5%)	2/34 (6%)	2/26 (8%)	5/25 (20%)
First incidence (days)	–	733 (T)	701	733 (T)	530
Poly-3 test	P < 0.001***	P = 0.218	P = 0.100	P = 0.193	P < 0.001***

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Stomach (Forestomach): Squamous Cell Carcinoma and Papilloma (Combined)					
Overall rate	0/47 (0%)	2/45 (4%)	3/48 (6%)	2/45 (4%)	12/41 (29%)
Adjusted rate	0.0%	4.7%	7.2%	5.3%	33.1%
Terminal rate	0/45 (0%)	2/41 (5%)	2/34 (6%)	2/26 (8%)	7/25 (28%)
First incidence (days)	–	733 (T)	701	733 (T)	530
Poly-3 test	P < 0.001***	P = 0.218	P = 0.100	P = 0.193	P < 0.001***
All Organs: Fibrous Histiocytoma					
Overall rate	2/48 (4%)	2/48 (4%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Adjusted rate	4.3%	4.4%	0.0%	0.0%	2.6%
Terminal rate	1/45 (2%)	1/41 (2%)	0/34 (0%)	0/26 (0%)	0/25 (0%)
First incidence (days)	694	701	–	–	586
Poly-3 test	P = 0.341N	P = 0.680	P = 0.264N	P = 0.283N	P = 0.567N
All Organs: Hemangioma					
Overall rate	0/48 (0%)	0/48 (0%)	1/48 (2%)	0/48 (0%)	1/48 (2%)
Adjusted rate	0.0%	0.0%	2.4%	0.0%	2.6%
Terminal rate	0/45 (0%)	0/41 (0%)	1/34 (3%)	0/26 (0%)	0/25 (0%)
First incidence (days)	–	–	733 (T)	–	659
Poly-3 test	P = 0.260	–	P = 0.477	–	P = 0.461
All Organs: Hemangiosarcoma					
Overall rate	2/48 (4%)	1/48 (2%)	2/48 (4%)	1/48 (2%)	3/48 (6%)
Adjusted rate	4.3%	2.2%	4.8%	2.6%	7.8%
Terminal rate	2/45 (4%)	1/41 (2%)	1/34 (3%)	1/26 (4%)	2/25 (8%)
First incidence (days)	733 (T)	733 (T)	716	733 (T)	669
Poly-3 test	P = 0.248	P = 0.513N	P = 0.653	P = 0.566N	P = 0.411
All Organs: Hemangiosarcoma or Hemangioma					
Overall rate	2/48 (4%)	1/48 (2%)	3/48 (6%)	1/48 (2%)	4/48 (8%)
Adjusted rate	4.3%	2.2%	7.2%	2.6%	10.3%
Terminal rate	2/45 (4%)	1/41 (2%)	2/34 (6%)	1/26 (4%)	2/25 (8%)
First incidence (days)	733 (T)	733 (T)	716	733 (T)	659
Poly-3 test	P = 0.135	P = 0.513N	P = 0.449	P = 0.566N	P = 0.255
All Organs: Histiocytic Sarcoma					
Overall rate	1/48 (2%)	0/48 (0%)	1/48 (2%)	3/48 (6%)	3/48 (6%)
Adjusted rate	2.1%	0.0%	2.4%	7.6%	7.7%
Terminal rate	1/45 (2%)	0/41 (0%)	0/34 (0%)	1/26 (4%)	1/25 (4%)
First incidence (days)	733 (T)	–	558	563	530
Poly-3 test	P = 0.042*	P = 0.507N	P = 0.738	P = 0.248	P = 0.244

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
All Organs: Leukemia (Lymphocytic, Monocytic, Mononuclear, or Undifferentiated)					
Overall rate	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	2.6%
Terminal rate	0/45 (0%)	0/41 (0%)	0/34 (0%)	0/26 (0%)	0/25 (0%)
First incidence (days)	–	–	–	–	472
Poly-3 test	P = 0.130	–	–	–	P = 0.463
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, NOS, or Undifferentiated Cell Type)					
Overall rate	2/48 (4%)	4/48 (8%)	2/48 (4%)	4/48 (8%)	7/48 (15%)
Adjusted rate	4.3%	8.6%	4.7%	10.0%	17.2%
Terminal rate	1/45 (2%)	2/41 (5%)	1/34 (3%)	1/26 (4%)	2/25 (8%)
First incidence (days)	715	400	604	413	388
Poly-3 test	P = 0.022*	P = 0.332	P = 0.655	P = 0.266	P = 0.049*
All Organs: Benign Tumors					
Overall rate	14/48 (29%)	23/48 (48%)	34/48 (71%)	40/48 (83%)	43/48 (90%)
Adjusted rate	29.9%	50.9%	76.0%	91.6%	95.4%
Terminal rate	14/45 (31%)	23/41 (56%)	25/34 (74%)	24/26 (93%)	25/25 (100%)
First incidence (days)	733 (T)	726 (T)	558	497	530
Poly-3 test	P < 0.001***	P = 0.030*	P < 0.001***	P < 0.001***	P < 0.001***
All Organs: Malignant Tumors					
Overall rate	13/48 (27%)	17/48 (35%)	21/48 (44%)	27/48 (56%)	33/48 (69%)
Adjusted rate	27.6%	35.6%	46.5%	59.4%	71.2%
Terminal rate	11/45 (24%)	11/41 (27%)	12/34 (35%)	11/26 (43%)	13/25 (52%)
First incidence (days)	694	400	387	352	388
Poly-3 test	P < 0.001***	P = 0.272	P = 0.047*	P = 0.001**	P < 0.001***
All Organs: Benign or Malignant Tumors					
Overall rate	23/48 (48%)	35/48 (73%)	41/48 (85%)	46/48 (96%)	47/48 (98%)
Adjusted rate	48.9%	73.3%	88.4%	98.8%	100.0%
Terminal rate	21/45 (47%)	29/41 (71%)	29/34 (85%)	26/26 (100%)	25/25 (100%)
First incidence (days)	694	400	387	352	388
Poly-3 test	P < 0.001***	P = 0.011*	P < 0.001***	P < 0.001***	P < 0.001***

[a]Number of animals with neoplasm per number of animals examined microscopically.
[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c]Observed incidence at the terminal sacrifice.
[d]T indicates terminal sacrifice.
[e]Beneath the 0 mM Glycidamide are the p-values associated with the trend test. Beneath the treated groups incidences are the p-values corresponding to pairwise comparisons between the 0 mM Glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.

Table C-3. Historical Incidence of Harderian Gland Neoplasms in NCTR Control Male B6C3F1/Nctr Mice

Study (Report Date)	Route of Administration	Incidence in Controls
		Adenoma
Sulfamethazine (February 1988)	Diet	15/184 (8.2%)
Doxylamine (April 1991)	Diet	1/48 (2.1%)
Triprolidine (June 1991)	Diet	0/48 (0.0%)
Pyrilamine (July 1991)	Diet	2/47 (4.3%)
Fumonisin B_1 (March 1999)	Diet	1/46 (2.2%)
Chloral Hydrate (July 2001)	Gavage	4/48 (8.3%)
Chloral Hydrate (October 2002)	Gavage	5/47 (10.6%)
Urethane and Ethanol (May 2003)	Drinking Water	3/47 (6.4%)
Acrylamide (February 2012)	Drinking Water	2/46 (4.3%)
Aloe vera Whole Leaf (2013)	Drinking Water	3/48 (6.3%)
Total (%) (All studies)		36/609 (5.9%)
Range		0.0–10.6%
Total (%) (Drinking water studies)		8/141 (5.7%)
Range		4.3–6.4%

Table C-4. Historical Incidence of Alveolar/Bronchiolar Neoplasms in NCTR Control Male B6C3F1/Nctr Mice

Study (Report Date)	Route of Administration	Incidence in Controls		
		Adenoma	Carcinoma	Adenoma or Carcinoma
Sulfamethazine (February 1988)	Diet	25/186 (13.4%)	3/186 (1.6%)	28/186 (15.1%)
Doxylamine (April 1991)	Diet	9/48 (18.8%)	0/48 (0.0%)	9/48 (18.8%)
Triprolidine (June 1991)	Diet	9/48 (18.8%)	2/48 (4.2%)	11/48 (22.9%)
Pyrilamine (July 1991)	Diet	5/47 (10.6%)	0/47 (0.0%)	5/47 (10.6%)
Fumonisin B_1 (March 1999)	Diet	6/48 (12.5%)	0/48 (0.0%)	6/48 (12.5%)
Chloral Hydrate (July 2001)	Gavage	4/48 (8.3%)	4/48 (8.3%)	8/48 (16.7%)
Chloral Hydrate (October 2002)	Gavage	13/48 (27.1%)	2/48 (4.2%)	15/48 (31.3%)
Urethane and Ethanol (May 2003)	Drinking Water	4/48 (8.3%)	1/48 (2.1%)	5/48 (10.4%)
Acrylamide (February 2012)	Drinking Water	5/47 (10.6%)	2/47 (4.3%)	6/47 (12.8%)
Aloe vera Whole Leaf (2013)	Drinking Water	3/48 (6.3%)	3/48 (6.3%)	6/48 (12.5%)
Total (%) (All studies)		83/616 (13.5%)	17/616 (2.8%)	99/616 (16.1%)
Range		6.3–27.1%	0.0–8.3%	10.4–31.3%
Total (%) (Drinking water studies)		12/143 (8.4%)	6/143 (4.2%)	17/143 (11.9%)
Range		6.3–10.6%	2.1–6.3%	10.4–12.8%

Table C-5. Historical Incidence of Squamous Cell Papilloma or Carcinoma (Combined) of the Forestomach in NCTR Control Male B6C3F1/Nctr Mice

Study (Report Date)	Route of Administration	Incidence in Controls
Sulfamethazine (February 1988)	Diet	1/179 (0.6%)
Doxylamine (April 1991)	Diet	0/47 (0.0%)
Triprolidine (June 1991)	Diet	0/48 (0.0%)
Pyrilamine (July 1991)	Diet	0/46 (0.0%)
Fumonisin B_1 (March 1999)	Diet	1/47 (2.1%)
Chloral Hydrate (July 2001)	Gavage	0/48 (0.0%)
Chloral Hydrate (October 2002)	Gavage	0/43 (0.0%)
Urethane and Ethanol (May 2003)	Drinking Water	0/46 (0.0%)
Acrylamide (February 2012)	Drinking Water	0/46 (0.0%)
Aloe vera Whole Leaf (2013)	Drinking Water	0/47 (0.0%)
Total (%) (All studies)		2/597 (0.3%)
Range		0.0–2.1%
Total (%) (Drinking water studies)		0/137 (0.0%)
Range		0.0%

Table C-6. Historical Incidence of Malignant Lymphoma in NCTR Control Male B6C3F1/Nctr Mice

Study (Report Date)	Route of Administration	Incidence in Controls
Sulfamethazine (February 1988)	Diet	10/187 (5.3%)
Doxylamine (April 1991)	Diet	5/48 (10.4%)
Triprolidine (June 1991)	Diet	3/48 (6.3%)
Pyrilamine (July 1991)	Diet	3/47 (6.4%)
Fumonisin B_1 (March 1999)	Diet	5/48 (10.4%)
Chloral Hydrate (July 2001)	Gavage	5/48 (10.4%)
Chloral Hydrate (October 2002)	Gavage	5/48 (10.4%)
Urethane and Ethanol (May 2003)	Drinking Water	9/48 (18.8%)
Acrylamide (February 2012)	Drinking Water	4/48 (8.3%)
Aloe vera Whole Leaf (2013)	Drinking Water	4/48 (8.3%)
Total (%) (All studies)		53/618 (8.6%)
Range		5.3–10.4%
Total (%) (Drinking water studies)		17/144 (11.8%)
Range		8.3–18.8%

Table C-7. Historical Incidence of Squamous Cell Papilloma or Carcinoma (Combined) of the Skin in NCTR Control Male B6C3F1/Nctr Mice

Study (Report Date)	Route of Administration	Incidence in Controls
Sulfamethazine (February 1988)	Diet	0/183 (0.0%)
Doxylamine (April 1991)	Diet	0/47 (0.0%)
Triprolidine (June 1991)	Diet	0/48 (0.0%)
Pyrilamine (July 1991)	Diet	0/47 (0.0%)
Fumonisin B_1 (March 1999)	Diet	0/48 (0.0%)
Chloral Hydrate (July 2001)	Gavage	0/47 (0.0%)
Chloral Hydrate (October 2002)	Gavage	0/48 (0.0%)
Urethane and Ethanol (May 2003)	Drinking Water	0/47 (0.0%)
Acrylamide (February 2012)	Drinking Water	1/47 (2.1%)
Aloe vera Whole Leaf (2013)	Drinking Water	0/48 (0.0%)
Total (%) (All studies)		1/610 (0.2%)
Range		0.0–2.1%
Total (%) (Drinking water studies)		1/42 (0.7%)
Range		0.0–2.1%

Table C-8. Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Two-year Drinking Water Study of Glycidamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Disposition Summary					
Animals initially in study	48	48	48	48	48
Early deaths					
Moribund sacrifice	–	4	11	16	11
Natural death	1	3	2	3	9
Survivors					
Moribund sacrifice	2	–	1	2	2
Natural death	–	–	–	1	1
Terminal sacrifice	45	41	34	26	25
Animals examined microscopically	48	48	48	48	48
Alimentary System					
Esophagus	(47)	(48)	(47)	(47)	(46)
Gallbladder	(46)	(45)	(46)	(45)	(39)
Infiltration cellular, lymphocyte	–	–	–	1 (2%)	–
Intestine large, cecum	(47)	(45)	(46)	(44)	(39)
Hyperplasia, lymphoid	1 (2%)	3 (7%)	4 (9%)	4 (9%)	2 (5%)
Intestine large, colon	(47)	(45)	(47)	(45)	(39)
Intestine large, rectum	(47)	(45)	(47)	(45)	(39)
Intestine small, duodenum	(47)	(45)	(46)	(44)	(39)
Epithelium, hyperplasia	–	–	–	–	1 (3%)
Intestine small, ileum	(47)	(45)	(46)	(44)	(39)
Hyperplasia, lymphoid	–	1 (2%)	–	–	2 (5%)
Intestine small, jejunum	(47)	(45)	(46)	(44)	(39)
Hyperplasia, lymphoid	1 (2%)	1 (2%)	–	–	1 (3%)
Epithelium, hyperplasia	–	–	1 (2%)	–	–
Liver	(47)	(47)	(47)	(47)	(48)
Angiectasis	–	–	–	–	1 (2%)
Basophilic focus	–	–	2 (4%)	4 (9%)	2 (4%)
Basophilic focus, multiple	–	–	–	–	1 (2%)
Cyst	–	–	–	1 (2%)	–
Eosinophilic focus	–	–	–	–	2 (4%)
Hematopoietic cell proliferation	2 (4%)	1 (2%)	2 (4%)	–	1 (2%)
Infiltration cellular, lymphocyte	2 (4%)	1 (2%)	1 (2%)	3 (6%)	–
Infiltration cellular, polymorphonuclear	–	–	–	–	1 (2%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Inflammation, suppurative	–	1 (2%)	–	–	–
Inflammation, chronic active	1 (2%)	3 (6%)	–	1 (2%)	1 (2%)
Mineralization	–	–	–	1 (2%)	–
Mixed cell focus	–	–	1 (2%)	–	–
Necrosis	–	3 (6%)	1 (2%)	3 (6%)	3 (6%)
Tension lipidosis	–	1 (2%)	–	1 (2%)	–
Vacuolization cytoplasmic	2 (4%)	–	1 (2%)	1 (2%)	–
Bile duct, hyperplasia	1 (2%)	–	–	–	–
Kupffer cell, hyperplasia	–	–	–	–	1 (2%)
Oval cell, hyperplasia	1 (2%)	–	–	–	1 (2%)
Mesentery	(2)	(1)	(0)	(1)	(1)
Hemorrhage	–	1 (100%)	–	–	–
Fat, necrosis	1 (50%)	–	–	1 (100%)	–
Oral mucosa	(0)	(1)	(0)	(0)	(2)
Pancreas	(47)	(46)	(47)	(47)	(45)
Cyst	–	–	–	1 (2%)	–
Infiltration cellular, lymphocyte	7 (15%)	3 (7%)	–	1 (2%)	3 (7%)
Acinus, degeneration	4 (9%)	1 (2%)	4 (9%)	6 (13%)	3 (7%)
Duct, dilatation	–	–	–	–	2 (4%)
Salivary glands	(47)	(47)	(47)	(46)	(44)
Infiltration cellular, lymphocyte	26 (55%)	22 (47%)	20 (43%)	17 (37%)	18 (41%)
Stomach, forestomach	(47)	(45)	(48)	(45)	(41)
Cyst epithelial inclusion	–	–	2 (4%)	–	–
Ulcer	–	1 (2%)	–	–	–
Epithelium, hyperplasia	5 (11%)	2 (4%)	5 (10%)	5 (11%)	12 (29%)
Serosa, necrosis	–	–	–	1 (2%)	–
Stomach, glandular	(47)	(45)	(46)	(44)	(38)
Epithelium, hyperplasia	–	1 (2%)	–	–	1 (3%)
Tongue	(0)	(0)	(0)	(1)	(0)
Cardiovascular System					
Blood vessel	(47)	(48)	(47)	(47)	(47)
Heart	(47)	(48)	(47)	(47)	(46)
Infiltration cellular, polymorphonuclear	–	–	–	–	1 (2%)
Endocrine System					
Adrenal cortex	(47)	(47)	(47)	(45)	(44)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Accessory adrenal cortical nodule	–	1 (2%)	2 (4%)	2 (4%)	–
Subcapsular, hyperplasia	43 (91%)	38 (81%)	45 (96%)	39 (87%)	36 (82%)
Adrenal Medulla	(47)	(46)	(47)	(45)	(43)
Hyperplasia	1 (2%)	–	–	–	–
Islets, pancreatic	(47)	(47)	(47)	(47)	(44)
Hyperplasia	1 (2%)	2 (4%)	–	2 (4%)	2 (5%)
Infiltration cellular, lymphocyte	–	–	–	1 (2%)	–
Parathyroid gland	(43)	(48)	(43)	(42)	(42)
Cyst	2 (5%)	–	1 (2%)	1 (2%)	2 (5%)
Pituitary gland	(45)	(44)	(47)	(42)	(42)
Pars distalis, cyst	1 (2%)	–	2 (4%)	–	1 (2%)
Thyroid gland	(47)	(48)	(47)	(44)	(43)
Cyst	–	–	1 (2%)	–	–
Infiltration cellular, lymphocyte	1 (2%)	–	–	–	–
Follicle, degeneration	2 (4%)	4 (8%)	2 (4%)	2 (5%)	4 (9%)
General Body System					
Tissue NOS	(0)	(0)	(0)	(1)	(1)
Genital System					
Epididymis	(47)	(47)	(47)	(46)	(43)
Exfoliated germ cell	–	–	1 (2%)	–	1 (2%)
Hypospermia	2 (4%)	–	3 (6%)	–	3 (7%)
Infiltration cellular, lymphocyte	–	1 (2%)	–	–	–
Infiltration cellular, polymorphonuclear	–	–	–	–	1 (2%)
Inflammation, chronic	–	1 (2%)	2 (4%)	–	3 (7%)
Inflammation, chronic active	1 (2%)	–	–	–	–
Spermatocele	–	–	–	–	1 (2%)
Penis	(0)	(0)	(1)	(0)	(0)
Inflammation, chronic active	–	–	1 (100%)	–	–
Ulcer	–	–	1 (100%)	–	–
Preputial gland	(47)	(47)	(46)	(46)	(44)
Cyst	3 (6%)	2 (4%)	5 (11%)	1 (2%)	4 (9%)
Degeneration	4 (9%)	10 (21%)	5 (11%)	12 (26%)	9 (20%)
Hyperkeratosis	1 (2%)	1 (2%)	1 (2%)	–	–
Infiltration cellular, lymphocyte	3 (6%)	–	1 (2%)	4 (9%)	1 (2%)
Inflammation, suppurative	–	5 (11%)	2 (4%)	3 (7%)	7 (16%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Inflammation, chronic active	1 (2%)	1 (2%)	–	–	2 (5%)
Duct, dilatation	–	–	1 (2%)	–	4 (9%)
Prostate	(47)	(47)	(47)	(45)	(41)
Inflammation, suppurative	–	–	1 (2%)	–	–
Inflammation, chronic active	–	–	–	–	1 (2%)
Epithelium, hyperplasia	–	–	–	–	1 (2%)
Seminal vesicle	(47)	(46)	(47)	(45)	(41)
Atrophy	–	–	–	1 (2%)	–
Lumen, dilatation	–	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Testes	(47)	(46)	(46)	(46)	(42)
Mineralization	–	–	–	1 (2%)	–
Seminiferous tubule, degeneration	2 (4%)	–	3 (7%)	2 (4%)	5 (12%)
Hematopoietic System					
Bone marrow	(47)	(47)	(47)	(46)	(44)
Hyperplasia	3 (6%)	3 (6%)	5 (11%)	5 (11%)	8 (18%)
Lymph node	(4)	(5)	(2)	(6)	(6)
Axillary, hyperplasia, lymphoid	–	1 (20%)	1 (50%)	–	1 (17%)
Iliac, hyperplasia, lymphoid	–	–	–	1 (17%)	1 (17%)
Iliac, infiltration cellular, polymorphonuclear	–	–	–	–	1 (17%)
Inguinal, hyperplasia, lymphoid	–	–	1 (50%)	2 (33%)	1 (17%)
Inguinal, infiltration cellular, plasma cell	–	–	–	–	1 (17%)
Lumbar, hyperplasia, lymphoid	1 (25%)	–	1 (50%)	–	–
Lumbar, infiltration cellular, polymorphonuclear	–	–	–	–	1 (17%)
Mediastinal, hyperplasia, lymphoid	1 (25%)	–	–	1 (17%)	–
Renal, hyperplasia, lymphoid	1 (25%)	–	–	–	1 (17%)
Renal, infiltration cellular, polymorphonuclear	–	–	–	–	1 (17%)
Lymph node, mandibular	(47)	(47)	(47)	(45)	(43)
Hyperplasia, lymphoid	7 (15%)	4 (9%)	5 (11%)	10 (22%)	7 (16%)
Infiltration cellular, histiocyte	–	–	–	1 (2%)	2 (5%)
Infiltration cellular, mast cell	–	–	1 (2%)	1 (2%)	2 (5%)
Infiltration cellular, plasma cell	1 (2%)	–	1 (2%)	3 (7%)	4 (9%)
Infiltration cellular, polymorphonuclear	–	–	–	–	1 (2%)
Pigmentation	–	–	1 (2%)	–	1 (2%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Sinus, dilatation	–	–	–	–	1 (2%)
Lymph node, mesenteric	(47)	(46)	(47)	(45)	(42)
Angiectasis	9 (19%)	5 (11%)	3 (6%)	5 (11%)	3 (7%)
Hematopoietic cell proliferation	–	–	–	–	4 (10%)
Hemorrhage	5 (11%)	12 (26%)	4 (9%)	4 (9%)	9 (21%)
Hyperplasia, lymphoid	25 (53%)	12 (26%)	21 (45%)	16 (36%)	12 (29%)
Infiltration cellular, histiocyte	5 (11%)	4 (9%)	3 (6%)	5 (11%)	5 (12%)
Infiltration cellular, mast cell	–	2 (4%)	1 (2%)	–	2 (5%)
Infiltration cellular, polymorphonuclear	1 (2%)	–	–	–	1 (2%)
Sinus, dilatation	–	1 (2%)	–	–	1 (2%)
Spleen	(47)	(47)	(47)	(46)	(44)
Congestion	–	–	–	–	1 (2%)
Depletion lymphoid	–	–	–	2 (4%)	–
Hematopoietic cell proliferation	6 (13%)	6 (13%)	12 (26%)	14 (30%)	17 (39%)
Hyperplasia, lymphoid	34 (72%)	31 (66%)	22 (47%)	22 (48%)	23 (52%)
Inflammation, granulomatous	–	–	1 (2%)	–	–
Necrosis	–	–	–	1 (2%)	–
Pigmentation	–	–	–	–	1 (2%)
Thymus	(43)	(45)	(43)	(39)	(34)
Atrophy	28 (65%)	25 (56%)	17 (40%)	21 (54%)	16 (47%)
Hyperplasia, lymphoid	–	–	1 (2%)	1 (3%)	–
Integumentary System					
Mammary gland	(3)	(1)	(0)	(2)	(1)
Skin	(47)	(48)	(47)	(47)	(46)
Fibrosis	–	1 (2%)	–	1 (2%)	–
Inflammation, chronic active	–	–	1 (2%)	1 (2%)	1 (2%)
Metaplasia, osseous	1 (2%)	–	–	–	–
Mineralization	–	–	–	1 (2%)	–
Ulcer	–	–	–	–	1 (2%)
Epithelium, hyperplasia	–	–	1 (2%)	–	1 (2%)
Sebaceous gland, hyperplasia	–	–	–	1 (2%)	–
Musculoskeletal System					
Bone, femur	(48)	(48)	(48)	(48)	(47)
Skeletal muscle	(48)	(47)	(47)	(47)	(47)
Degeneration	–	–	–	1 (2%)	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Nervous System					
Brain, brain stem	(47)	(47)	(46)	(46)	(42)
Brain, cerebellum	(47)	(47)	(46)	(46)	(42)
Hemorrhage	–	–	1 (2%)	–	–
Brain, cerebrum	(47)	(47)	(46)	(46)	(42)
Cyst	–	1 (2%)	–	–	–
Mineralization	33 (70%)	30 (64%)	32 (70%)	29 (63%)	20 (48%)
Meninges, arteritis	1 (2%)	–	–	–	–
Peripheral nerve, sciatic	(47)	(47)	(47)	(46)	(42)
Axon, degeneration	36 (77%)	37 (79%)	30 (64%)	30 (65%)	32 (76%)
Spinal cord, cervical	(47)	(46)	(46)	(44)	(39)
Axon, degeneration	8 (17%)	8 (17%)	3 (7%)	11 (25%)	12 (31%)
Nerve, degeneration	–	–	1 (2%)	–	–
Spinal cord, lumbar	(47)	(46)	(46)	(45)	(39)
Axon, degeneration	9 (19%)	10 (22%)	4 (9%)	–	1 (3%)
Nerve, degeneration	39 (83%)	41 (89%)	37 (80%)	35 (78%)	34 (87%)
Spinal cord, thoracic	(47)	(46)	(46)	(45)	(39)
Axon, degeneration	37 (79%)	28 (61%)	23 (50%)	24 (53%)	26 (67%)
Nerve, degeneration	1 (2%)	1 (2%)	1 (2%)	–	–
Respiratory System					
Lung	(47)	(46)	(47)	(47)	(47)
Congestion	–	–	1 (2%)	–	–
Hemorrhage	–	–	–	–	1 (2%)
Infiltration cellular, histiocyte	–	1 (2%)	–	1 (2%)	4 (9%)
Infiltration cellular, lymphocyte	–	–	1 (2%)	–	–
Inflammation, chronic	–	–	–	–	1 (2%)
Alveolar epithelium, hyperplasia	–	1 (2%)	4 (9%)	3 (6%)	6 (13%)
Alveolus, infiltration cellular, histiocyte	–	–	–	–	1 (2%)
Nose	(47)	(46)	(47)	(47)	(45)
Amyloid deposition	–	–	1 (2%)	–	–
Crystals	–	–	2 (4%)	1 (2%)	1 (2%)
Foreign body	–	–	–	1 (2%)	–
Hyaline droplet	13 (28%)	6 (13%)	11 (23%)	2 (4%)	1 (2%)
Inflammation, suppurative	–	1 (2%)	–	1 (2%)	1 (2%)
Trachea	(47)	(48)	(46)	(46)	(45)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Special Senses System					
Eye	(47)	(45)	(46)	(44)	(42)
Cataract	1 (2%)	3 (7%)	7 (15%)	8 (18%)	17 (40%)
Cornea, inflammation, chronic	–	–	–	–	1 (2%)
Cornea, inflammation, chronic active	–	–	2 (4%)	–	7 (17%)
Cornea, ulcer	–	–	1 (2%)	–	3 (7%)
Harderian gland	(47)	(47)	(47)	(46)	(47)
Cyst	–	–	–	–	1 (2%)
Infiltration cellular, lymphocyte	4 (9%)	–	1 (2%)	–	–
Epithelium, hyperplasia	–	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Zymbal's gland	(0)	(0)	(0)	(0)	(1)
Keratin cyst	–	–	–	–	1 (100%)
Urinary System					
Kidney	(47)	(47)	(47)	(46)	(46)
Autolysis	–	–	–	1 (2%)	1 (2%)
Cyst	1 (2%)	–	–	–	–
Degeneration	–	–	–	–	1 (2%)
Hyaline droplet	–	–	1 (2%)	3 (7%)	2 (4%)
Hydronephrosis	1 (2%)	–	–	–	1 (2%)
Infarct	1 (2%)	–	–	1 (2%)	1 (2%)
Infiltration cellular, lymphocyte	20 (43%)	12 (26%)	19 (40%)	8 (17%)	9 (20%)
Infiltration cellular, polymorphonuclear	–	–	–	–	1 (2%)
Inflammation, chronic	1 (2%)	–	–	–	–
Nephropathy	15 (32%)	18 (38%)	14 (30%)	12 (26%)	16 (35%)
Urinary bladder	(47)	(46)	(47)	(45)	(41)
Infiltration cellular, lymphocyte	7 (15%)	4 (9%)	3 (6%)	3 (7%)	1 (2%)
Infiltration cellular, polymorphonuclear	–	–	–	–	1 (2%)
Inflammation, suppurative	–	–	–	–	1 (2%)
Lumen, dilatation	3 (6%)	2 (4%)	3 (6%)	1 (2%)	2 (5%)

[a]Number of animals examined microscopically at the site and the number of animals with lesion.

Appendix D. Summary of Lesions in Female Mice in the Two-year Drinking Water Study of Glycidamide

Tables

Table D-1. Summary of the Incidence of Neoplasms in Female Mice in the Two-year Drinking Water Study of Glycidamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Disposition Summary					
Animals initially in study	48	48	48	48	48
Early deaths					
Moribund sacrifice	2	2	6	7	32
Natural deaths	1	2	2	4	6
Survivors					
Moribund sacrifice	2	–	2	5	2
Natural deaths	2	2	–	1	–
Terminal sacrifice	41	42	38	31	8
Animals examined microscopically	47	48	48	48	48
Alimentary System					
Esophagus	(46)	(48)	(46)	(47)	(45)
Squamous cell carcinoma	–	–	–	1 (2%)	–
Gallbladder	(45)	(44)	(47)	(44)	(41)
Lymphoma malignant	1 (2%)	–	–	1 (2%)	–
Intestine large, cecum	(45)	(44)	(46)	(45)	(42)
Lymphoma malignant	–	–	1 (2%)	1 (2%)	1 (2%)
Intestine large, colon	(45)	(45)	(47)	(45)	(43)
Lymphoma malignant	–	–	–	–	1 (2%)
Intestine large, rectum	(45)	(45)	(46)	(45)	(43)
Polyp adenomatous	–	–	–	–	1 (2%)
Sarcoma stromal, metastatic, uterus	–	–	–	–	1 (2%)
Intestine small, duodenum	(45)	(44)	(46)	(45)	(42)
Adenoma	–	–	–	–	1 (2%)
Intestine small, ileum	(45)	(44)	(46)	(45)	(42)
Fibrous histiocytoma	1 (2%)	–	–	–	–
Lymphoma malignant	–	1 (2%)	–	–	–
Intestine small, jejunum	(45)	(44)	(46)	(45)	(42)
Lymphoma malignant	–	–	1 (2%)	–	–
Liver	(47)	(48)	(47)	(46)	(43)
Fibrous histiocytoma	1 (2%)	–	–	–	–
Hemangiosarcoma	–	–	1 (2%)	–	–
Hepatocellular adenoma	4 (9%)	2 (4%)	4 (9%)	2 (4%)	–
Hepatocellular carcinoma	–	1 (2%)	2 (4%)	2 (4%)	1 (2%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Histiocytic sarcoma	1 (2%)	3 (6%)	–	1 (2%)	4 (9%)
Leukemia	1 (2%)	–	–	–	–
Lymphoma malignant	2 (4%)	2 (4%)	3 (6%)	1 (2%)	4 (9%)
Pancreas	(45)	(48)	(47)	(46)	(44)
Fibrous histiocytoma	1 (2%)	–	–	–	–
Lymphoma malignant	2 (4%)	1 (2%)	2 (4%)	1 (2%)	3 (7%)
Salivary glands	(45)	(47)	(47)	(47)	(44)
Lymphoma malignant	1 (2%)	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Stomach, forestomach	(45)	(45)	(47)	(45)	(44)
Lymphoma malignant	–	–	1 (2%)	–	–
Squamous cell papilloma	1 (2%)	1 (2%)	1 (2%)	5 (11%)	9 (20%)
Stomach, glandular	(45)	(45)	(47)	(45)	(43)
Lymphoma malignant	–	–	–	–	1 (2%)
Cardiovascular System					
Blood vessel	(46)	(48)	(47)	(47)	(45)
Lymphoma malignant	–	–	–	–	1 (2%)
Heart	(46)	(48)	(47)	(47)	(45)
Histiocytic sarcoma	–	1 (2%)	–	–	1 (2%)
Leukemia	1 (2%)	–	–	–	–
Lymphoma malignant	–	–	1 (2%)	–	–
Endocrine System					
Adrenal cortex	(45)	(48)	(47)	(47)	(44)
Histiocytic sarcoma	–	–	–	1 (2%)	–
Lymphoma malignant	–	–	–	–	3 (7%)
Subcapsular, adenoma	–	–	–	–	1 (2%)
Adrenal medulla	(45)	(45)	(47)	(47)	(43)
Pheochromocytoma benign	–	–	–	1 (2%)	–
Pheochromocytoma complex	–	–	–	–	1 (2%)
Pheochromocytoma malignant	–	1 (2%)	–	–	–
Islets, pancreatic	(45)	(48)	(47)	(46)	(43)
Lymphoma malignant	–	–	–	1 (2%)	–
Parathyroid gland	(41)	(45)	(46)	(40)	(39)
Pituitary gland	(45)	(45)	(43)	(47)	(43)
Histiocytic sarcoma	–	1 (2%)	1 (2%)	–	–
Lymphoma malignant	–	–	–	–	1 (2%)
Pars distalis, adenoma	–	2 (4%)	–	1 (2%)	2 (5%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Thyroid gland	(45)	(47)	(46)	(46)	(44)
General Body System					
Tissue NOS	(0)	(0)	(0)	(0)	(1)
Lymphoma malignant	–	–	–	–	1 (100%)
Genital System					
Clitoral gland	(44)	(48)	(47)	(47)	(41)
Lymphoma malignant	–	–	–	–	1 (2%)
Schwannoma malignant, metastatic, skin	–	–	–	–	1 (2%)
Squamous cell carcinoma, metastatic, vagina	–	–	–	–	1 (2%)
Ovary	(45)	(47)	(47)	(46)	(44)
Granulosa cell tumor benign	–	–	–	1 (2%)	2 (5%)
Granulosa cell tumor malignant	–	–	–	2 (4%)	1 (2%)
Histiocytic sarcoma	–	1 (2%)	–	1 (2%)	–
Lymphoma malignant	–	–	–	1 (2%)	3 (7%)
Tubulostromal adenoma	–	–	–	1 (2%)	2 (5%)
Uterus	(45)	(48)	(47)	(47)	(45)
Adenocarcinoma	–	–	–	–	2 (4%)
Hemangioma	–	–	–	1 (2%)	–
Hemangiosarcoma	–	–	–	1 (2%)	1 (2%)
Histiocytic sarcoma	–	1 (2%)	2 (4%)	1 (2%)	–
Lymphoma malignant	–	–	1 (2%)	–	3 (7%)
Polyp stromal	1 (2%)	1 (2%)	–	3 (6%)	4 (9%)
Sarcoma stromal	–	–	–	–	2 (4%)
Endometrium, adenoma	–	–	1 (2%)	–	1 (2%)
Vagina	(0)	(0)	(0)	(0)	(1)
Squamous cell carcinoma	–	–	–	–	1 (100%)
Hematopoietic System					
Bone marrow	(46)	(47)	(47)	(45)	(43)
Hemangiosarcoma	1 (2%)	–	–	–	–
Hemangiosarcoma, metastatic, liver	–	–	1 (2%)	–	–
Histiocytic sarcoma	1 (2%)	–	–	1 (2%)	2 (5%)
Leukemia	1 (2%)	–	–	–	–
Lymphoma malignant	–	–	–	–	1 (2%)
Lymph node	(8)	(2)	(5)	(8)	(12)
Axillary, lymphoma malignant	–	–	–	1 (13%)	2 (17%)
Brachial, lymphoma malignant	–	–	–	–	1 (8%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Inguinal, lymphoma malignant	1 (13%)	–	–	1 (13%)	1 (8%)
Lumbar, lymphoma malignant	2 (25%)	1 (50%)	3 (60%)	1 (13%)	3 (25%)
Lumbar, squamous cell carcinoma, metastatic, vagina	–	–	–	–	1 (8%)
Mediastinal, histiocytic sarcoma	1 (13%)	–	–	–	2 (17%)
Mediastinal, lymphoma malignant	1 (13%)	–	1 (20%)	–	2 (17%)
Pancreatic, fibrous histiocytoma	1 (13%)	–	–	–	–
Pancreatic, histiocytic sarcoma	–	–	–	–	1 (8%)
Pancreatic, lymphoma malignant	2 (25%)	1 (50%)	–	2 (25%)	1 (8%)
Popliteal, lymphoma malignant	–	–	–	–	1 (8%)
Renal, fibrous histiocytoma	1 (13%)	–	–	–	–
Renal, histiocytic sarcoma	–	–	–	1 (13%)	1 (8%)
Renal, leukemia	1 (13%)	–	–	–	–
Renal, lymphoma malignant	–	1 (50%)	2 (40%)	4 (50%)	1 (8%)
Lymph node, mandibular	(46)	(48)	(47)	(47)	(43)
Histiocytic sarcoma	–	2 (4%)	–	1 (2%)	3 (7%)
Leukemia	1 (2%)	–	–	–	–
Lymphoma malignant	3 (7%)	3 (6%)	4 (9%)	4 (9%)	5 (12%)
Lymph node, mesenteric	(43)	(46)	(46)	(47)	(43)
Fibrous histiocytoma	1 (2%)	–	–	–	–
Histiocytic sarcoma	1 (2%)	2 (4%)	–	1 (2%)	4 (9%)
Lymphoma malignant	4 (9%)	4 (9%)	5 (11%)	5 (11%)	5 (12%)
Spleen	(46)	(47)	(47)	(47)	(45)
Fibrous histiocytoma	1 (2%)	–	–	–	–
Hemangiosarcoma	1 (2%)	–	–	1 (2%)	1 (2%)
Histiocytic sarcoma	2 (4%)	3 (6%)	–	1 (2%)	4 (9%)
Lymphoma malignant	8 (17%)	4 (9%)	4 (9%)	5 (11%)	6 (13%)
Thymus	(43)	(44)	(45)	(43)	(37)
Histiocytic sarcoma	–	1 (2%)	–	1 (2%)	1 (3%)
Leukemia	1 (2%)	–	–	–	–
Lymphoma malignant	2 (5%)	3 (7%)	4 (9%)	3 (7%)	3 (8%)
Integumentary System					
Mammary gland	(45)	(48)	(47)	(47)	(45)
Adenoacanthoma	–	–	–	1 (2%)	8 (18%)
Adenocarcinoma	1 (2%)	1 (2%)	2 (4%)	9 (19%)	11 (24%)
Lymphoma malignant	–	–	1 (2%)	–	2 (4%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Skin	(45)	(48)	(47)	(47)	(45)
Lymphoma malignant	–	–	1 (2%)	–	1 (2%)
Squamous cell carcinoma	–	–	–	1 (2%)	–
Squamous cell papilloma	–	–	1 (2%)	1 (2%)	–
Ear, basosquamous tumor malignant	–	–	–	–	1 (2%)
Subcutaneous tissue, fibrosarcoma	–	–	2 (4%)	2 (4%)	8 (18%)
Subcutaneous tissue, fibrosarcoma, multiple	–	1 (2%)	–	–	1 (2%)
Subcutaneous tissue, fibrous histiocytoma	–	–	–	1 (2%)	–
Subcutaneous tissue, sarcoma	–	–	1 (2%)	3 (6%)	3 (7%)
Subcutaneous tissue, Schwannoma malignant	–	–	1 (2%)	1 (2%)	1 (2%)
Musculoskeletal System					
Bone	(0)	(0)	(1)	(2)	(1)
Cranium, rhabdomyosarcoma, metastatic, skeletal muscle	–	–	–	1 (50%)	–
Vertebra, osteosarcoma	–	–	–	–	1 (100%)
Bone, femur	(46)	(48)	(48)	(48)	(48)
Skeletal muscle	(46)	(48)	(47)	(47)	(45)
Fibrosarcoma, metastatic, skin	–	–	–	–	1 (2%)
Lymphoma malignant	–	–	–	–	1 (2%)
Rhabdomyosarcoma	–	–	–	1 (2%)	–
Sarcoma, metastatic, skin	–	–	–	–	1 (2%)
Nervous System					
Brain, brain stem	(45)	(47)	(47)	(45)	(44)
Histiocytic sarcoma	–	–	1 (2%)	–	–
Brain, cerebellum	(45)	(47)	(47)	(45)	(44)
Histiocytic sarcoma	–	–	1 (2%)	–	–
Brain, cerebrum	(45)	(47)	(47)	(45)	(44)
Histiocytic sarcoma	–	–	1 (2%)	–	–
Peripheral nerve, sciatic	(45)	(47)	(47)	(45)	(44)
Lymphoma malignant	–	–	–	–	1 (2%)
Spinal cord, cervical	(45)	(44)	(47)	(45)	(43)
Histiocytic sarcoma	–	–	1 (2%)	–	–
Spinal cord, lumbar	(45)	(45)	(47)	(45)	(43)
Histiocytic sarcoma	–	–	1 (2%)	–	–
Lymphoma malignant	–	–	–	–	1 (2%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Spinal cord, thoracic	(45)	(44)	(47)	(45)	(43)
Histiocytic sarcoma	–	–	1 (2%)	–	–
Respiratory System					
Lung	(46)	(48)	(47)	(47)	(44)
Alveolar/bronchiolar adenoma	3 (7%)	5 (10%)	3 (6%)	7 (15%)	7 (16%)
Alveolar/bronchiolar adenoma, multiple	–	–	–	–	2 (5%)
Alveolar/bronchiolar carcinoma	1 (2%)	1 (2%)	–	1 (2%)	2 (5%)
Fibrosarcoma, metastatic, skin	–	–	–	–	1 (2%)
Granular cell tumor malignant, metastatic, ovary	–	–	–	–	1 (2%)
Histiocytic sarcoma	1 (2%)	2 (4%)	–	1 (2%)	4 (9%)
Leukemia	1 (2%)	–	–	–	–
Lymphoma malignant	2 (4%)	2 (4%)	2 (4%)	1 (2%)	4 (9%)
Osteosarcoma, metastatic, bone	–	–	–	–	1 (2%)
Nose	(46)	(46)	(47)	(46)	(45)
Rhabdomyosarcoma, metastatic, skeletal muscle	–	–	–	1 (2%)	–
Trachea	(45)	(47)	(46)	(46)	(43)
Special Senses System					
Eye	(45)	(44)	(47)	(44)	(43)
Lymphoma malignant	–	–	1 (2%)	–	–
Rhabdomyosarcoma, metastatic, skeletal muscle	–	–	–	1 (2%)	–
Harderian gland	(45)	(47)	(47)	(46)	(46)
Adenocarcinoma	–	–	1 (2%)	–	–
Adenoma	2 (4%)	18 (38%)	17 (36%)	17 (37%)	27 (59%)
Bilateral, adenoma	–	1 (2%)	3 (6%)	7 (15%)	13 (28%)
Urinary System					
Kidney	(46)	(47)	(47)	(46)	(43)
Histiocytic sarcoma	–	2 (4%)	–	1 (2%)	–
Leukemia	1 (2%)	–	–	–	–
Lymphoma malignant	2 (4%)	3 (6%)	2 (4%)	1 (2%)	4 (9%)
Urinary bladder	(45)	(46)	(47)	(46)	(42)
Lymphoma malignant	1 (2%)	3 (7%)	2 (4%)	1 (2%)	1 (2%)
Sarcoma stromal, metastatic, uterus	–	–	–	–	1 (2%)
Systemic Lesions					
Multiple organs	(47)[b]	(48)[b]	(48)[b]	(48)[b]	(48)[b]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Histiocytic sarcoma	2 (4%)	3 (6%)	3 (6%)	1 (2%)	4 (8%)
Leukemia	1 (2%)	–	–	–	–
Lymphoma malignant	8 (17%)	5 (10%)	7 (15%)	5 (10%)	6 (13%)
Neoplasm Summary					
Total animals with primary neoplasms[c]	23	35	34	41	44
Total primary neoplasms	33	43	50	80	128
Total animals with benign neoplasms	10	26	27	35	42
Total benign neoplasms	11	30	30	47	72
Total animals with malignant neoplasms	15	13	19	25	37
Total malignant neoplasms	22	13	20	33	56
Total animals with metastatic neoplasms	–	–	1	1	8
Total metastatic neoplasms	–	–	1	3	10

[a]Number of animals examined microscopically at the site and the number of animals with neoplasm.
[b]Number of animals with any tissue examined microscopically.
[c]Primary neoplasms: all neoplasms except metastatic neoplasms.

Table D-2. Statistical Analysis of Neoplasms in Female Mice in the Two-year Drinking Water Study of Glycidamide

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Adrenal Medulla: Benign Pheochromocytoma					
Overall rate[a]	0/45 (0%)	0/45 (0%)	0/47 (0%)	1/47 (2%)	0/43 (0%)
Adjusted rate[b]	0.0%	0.0%	0.0%	2.4%	0.0%
Terminal rate[c]	0/41 (0%)	0/42 (0%)	0/38 (0%)	0/31 (0%)	0/8 (0%)
First incidence (days)[d]	–	–	–	644	–
Poly-3 test[e]	P = 0.363	–	–	P = 0.491	–
Adrenal Medulla: Malignant Pheochromocytoma					
Overall rate	0/45 (0%)	1/45 (2%)	0/47 (0%)	0/47 (0%)	0/43 (0%)
Adjusted rate	0.0%	2.3%	0.0%	0.0%	0.0%
Terminal rate	0/41 (0%)	1/42 (2%)	0/38 (0%)	0/31 (0%)	0/8 (0%)
First incidence (days)	–	733 (T)	–	–	–
Poly-3 test	P = 0.450N	P = 0.499	–	–	–
Adrenal Medulla: Benign and Malignant Pheochromocytoma (Combined)					
Overall rate	0/45 (0%)	1/45 (2%)	0/47 (0%)	1/47 (2%)	0/43 (0%)
Adjusted rate	0.0%	2.3%	0.0%	2.4%	0.0%
Terminal rate	0/41 (0%)	1/42 (2%)	0/38 (0%)	0/31 (0%)	0/8 (0%)
First incidence (days)	–	733 (T)	–	644	–
Poly-3 test	P = 0.567	P = 0.499	–	P = 0.491	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Harderian Gland: Adenoma					
Overall rate	2/45 (4%)	19/47 (40%)	20/47 (43%)	24/46 (52%)	40/46 (87%)
Adjusted rate	4.5%	41.2%	44.0%	54.9%	93.4%
Terminal rate	1/41 (2%)	17/42 (41%)	15/38 (40%)	15/31 (48%)	7/8 (88%)
First incidence (days)	636	537	519	512	394
Poly-3 test	P < 0.001***	P < 0.001***	P < 0.001***	P < 0.001***	P < 0.001***
Harderian Gland: Bilateral Adenoma					
Overall rate	0/45 (0%)	1/47 (2%)	3/47 (6%)	7/46 (15%)	13/46 (28%)
Adjusted rate	0.0%	2.2%	6.9%	16.9%	40.4%
Terminal rate	0/41 (0%)	1/42 (2%)	3/38 (8%)	5/31 (16%)	2/8 (25%)
First incidence (days)	–	733 (T)	733 (T)	667	498
Poly-3 test	P < 0.001***	P = 0.504	P = 0.117	P = 0.006**	P < 0.001***
Liver: Hepatocellular Adenoma					
Overall rate	4/47 (9%)	2/48 (4%)	4/47 (9%)	2/46 (4%)	0/43 (0%)
Adjusted rate	8.8%	4.4%	9.1%	4.9%	0.0%
Terminal rate	3/41 (7%)	2/42 (5%)	3/38 (8%)	2/31 (7%)	0/8 (0%)
First incidence (days)	722	733 (T)	541	733 (T)	–
Poly-3 test	P = 0.143N	P = 0.341N	P = 0.625	P = 0.393N	P = 0.176N
Liver: Hepatocellular Carcinoma					
Overall rate	0/47 (0%)	1/48 (2%)	2/47 (4%)	2/46 (4%)	1/43 (2%)
Adjusted rate	0.0%	2.2%	4.5%	4.9%	3.9%
Terminal rate	0/41 (0%)	0/42 (0%)	1/38 (3%)	1/31 (3%)	0/8 (0%)
First incidence (days)	–	704	586	726	647
Poly-3 test	P = 0.184	P = 0.499	P = 0.230	P = 0.212	P = 0.391
Liver: Hepatocellular Adenoma and Carcinoma (Combined)					
Overall rate	4/47 (9%)	3/48 (6%)	6/47 (13%)	4/46 (9%)	1/43 (2%)
Adjusted rate	8.8%	6.6%	13.4%	9.9%	3.9%
Terminal rate	3/41 (7%)	2/42 (5%)	4/38 (11%)	3/31 (10%)	0/8 (0%)
First incidence (days)	722	704	541	726	647
Poly-3 test	P = 0.406N	P = 0.503N	P = 0.356	P = 0.577	P = 0.395N
Lung: Alveolar/Bronchiolar Adenoma					
Overall rate	3/46 (7%)	5/48 (10%)	3/47 (6%)	7/47 (15%)	9/44 (21%)
Adjusted rate	6.7%	11.0%	6.9%	16.4%	31.0%
Terminal rate	2/41 (5%)	5/42 (12%)	3/38 (8%)	2/31 (7%)	1/8 (13%)
First incidence (days)	722	733 (T)	733 (T)	512	498
Poly-3 test	P = 0.002**	P = 0.363	P = 0.650	P = 0.139	P = 0.007**

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Lung: Alveolar/Bronchiolar Carcinoma					
Overall rate	1/46 (2%)	1/48 (2%)	0/47 (0%)	1/47 (2%)	2/44 (5%)
Adjusted rate	2.2%	2.2%	0.0%	2.4%	7.6%
Terminal rate	1/41 (2%)	0/42 (0%)	0/38 (0%)	1/31 (3%)	1/8 (13%)
First incidence (days)	733 (T)	540	–	733 (T)	659
Poly-3 test	P = 0.166	P = 0.755N	P = 0.505N	P = 0.744	P = 0.328
Lung: Alveolar/Bronchiolar Adenoma and Carcinoma (Combined)					
Overall rate	4/46 (9%)	6/48 (13%)	3/47 (6%)	8/47 (17%)	11/44 (25%)
Adjusted rate	8.9%	13.1%	6.9%	18.7%	37.5%
Terminal rate	3/41 (7%)	5/42 (12%)	3/38 (8%)	3/31 (10%)	2/8 (25%)
First incidence (days)	722	540	733 (T)	512	498
Poly-3 test	P < 0.001***	P = 0.385	P = 0.514N	P = 0.155	P = 0.003**
Mammary Gland: Adenoacanthoma					
Overall rate	0/45 (0%)	0/48 (0%)	0/47 (0%)	1/47 (2%)	8/45 (18%)
Adjusted rate	0.0%	0.0%	0.0%	2.4%	27.2%
Terminal rate	0/41 (0%)	0/42 (0%)	0/38 (0%)	1/31 (3%)	1/8 (13%)
First incidence (days)	–	–	–	733 (T)	498
Poly-3 test	P < 0.001***	–	–	P = 0.489	P < 0.001***
Mammary Gland: Adenocarcinoma					
Overall rate	1/45 (2%)	1/48 (2%)	2/47 (4%)	9/47 (19%)	11/45 (24%)
Adjusted rate	2.3%	2.2%	4.6%	21.4%	36.8%
Terminal rate	0/41 (0%)	1/42 (2%)	2/38 (5%)	5/31 (16%)	2/8 (25%)
First incidence (days)	688	733 (T)	733 (T)	622	477
Poly-3 test	P < 0.001***	P = 0.755N	P = 0.496	P = 0.006**	P < 0.001***
Mammary Gland: Adenoacanthoma and Adenocarcinoma (Combined)					
Overall rate	1/45 (2%)	1/48 (2%)	2/47 (4%)	9/47 (19%)	18/45 (40%)
Adjusted rate	2.3%	2.2%	4.6%	21.4%	54.7%
Terminal rate	0/41 (0%)	1/42 (2%)	2/38 (5%)	5/31 (16%)	3/8 (38%)
First incidence (days)	688	733 (T)	733 (T)	622	477
Poly-3 test	P < 0.001***	P = 0.755N	P = 0.496	P = 0.006**	P < 0.001***
Ovary: Benign Granulosa Cell Tumor					
Overall rate	0/45 (0%)	0/47 (0%)	0/47 (0%)	1/46 (2%)	2/44 (5%)
Adjusted rate	0.0%	0.0%	0.0%	2.4%	7.5%
Terminal rate	0/41 (0%)	0/41 (0%)	0/38 (0%)	0/31 (0%)	1/8 (13%)
First incidence (days)	–	–	–	674	576
Poly-3 test	P = 0.014*	–	–	P = 0.487	P = 0.146

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Skin: Fibrosarcoma					
Overall rate	0/45 (0%)	1/48 (2%)	2/47 (4%)	2/47 (4%)	9/45 (20%)
Adjusted rate	0.0%	2.2%	4.6%	4.7%	30.4%
Terminal rate	0/41 (0%)	1/42 (2%)	1/38 (3%)	0/31 (0%)	1/8 (13%)
First incidence (days)	–	733 (T)	701	520	421
Poly-3 test	P < 0.001***	P = 0.506	P = 0.236	P = 0.229	P < 0.001***
Skin: Sarcoma					
Overall rate	0/45 (0%)	0/48 (0%)	1/47 (2%)	3/47 (6%)	3/45 (7%)
Adjusted rate	0.0%	0.0%	2.3%	7.2%	10.7%
Terminal rate	0/41 (0%)	0/42 (0%)	0/38 (0%)	1/31 (3%)	0/8 (0%)
First incidence (days)	–	–	707	667	384
Poly-3 test	P = 0.004**	–	P = 0.499	P = 0.110	P = 0.059
Skin: Fibrosarcoma and Sarcoma (Combined)					
Overall rate	0/45 (0%)	1/48 (2%)	3/47 (6%)	5/47 (11%)	12/45 (27%)
Adjusted rate	0.0%	2.2%	6.8%	11.7%	38.2%
Terminal rate	0/41 (0%)	1/42 (2%)	1/38 (3%)	1/31 (3%)	1/8 (13%)
First incidence (days)	–	733 (T)	701	520	384
Poly-3 test	P < 0.001***	P = 0.506	P = 0.118	P = 0.028*	P < 0.001***
Skin: Squamous Cell Carcinoma					
Overall rate	0/45 (0%)	0/48 (0%)	0/47 (0%)	1/47 (2%)	0/45 (0%)
Adjusted rate	0.0%	0.0%	0.0%	2.4%	0.0%
Terminal rate	0/41 (0%)	0/42 (0%)	0/38 (0%)	1/31 (3%)	0/8 (0%)
First incidence (days)	–	–	–	733 (T)	–
Poly-3 test	P = 0.365	–	–	P = 0.489	–
Skin: Squamous Cell Papilloma					
Overall rate	0/45 (0%)	0/48 (0%)	1/47 (2%)	1/47 (2%)	0/45 (0%)
Adjusted rate	0.0%	0.0%	2.3%	2.4%	0.0%
Terminal rate	0/41 (0%)	0/42 (0%)	1/38 (3%)	1/31 (3%)	0/8 (0%)
First incidence (days)	–	–	733 (T)	733 (T)	–
Poly-3 test	P = 0.431	–	P = 0.499	P = 0.489	–
Skin: Squamous Cell Carcinoma and Papilloma (Combined)					
Overall rate	0/45 (0%)	0/48 (0%)	1/47 (2%)	2/47 (4%)	0/45 (0%)
Adjusted rate	0.0%	0.0%	2.3%	4.8%	0.0%
Terminal rate	0/41 (0%)	0/42 (0%)	1/38 (3%)	2/31 (7%)	0/8 (0%)
First incidence (days)	–	–	733 (T)	733 (T)	–
Poly-3 test	P = 0.271	–	P = 0.499	P = 0.225	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Stomach (Forestomach): Squamous Cell Papilloma					
Overall rate	1/45 (2%)	1/45 (2%)	1/47 (2%)	5/45 (11%)	9/44 (21%)
Adjusted rate	2.3%	2.3%	2.3%	12.2%	30.7%
Terminal rate	1/41 (2%)	1/42 (2%)	1/38 (3%)	4/31 (13%)	2/8 (25%)
First incidence (days)	733 (T)	733 (T)	733 (T)	379	384
Poly-3 test	P < 0.001***	P = 0.759	P = 0.759	P = 0.086	P < 0.001***
Uterus: Stromal Polyp					
Overall rate	1/45 (2%)	1/48 (2%)	0/47 (0%)	3/47 (6%)	4/45 (9%)
Adjusted rate	2.3%	2.2%	0.0%	7.1%	15.0%
Terminal rate	0/41 (0%)	1/42 (2%)	0/38 (0%)	2/31 (7%)	3/8 (38%)
First incidence (days)	688	733 (T)	–	520	645
Poly-3 test	P = 0.007**	P = 0.755N	P = 0.502N	P = 0.290	P = 0.070
Uterus: Stromal Sarcoma					
Overall rate	0/45 (0%)	0/48 (0%)	0/47 (0%)	0/47 (0%)	2/45 (4%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	7.3%
Terminal rate	0/41 (0%)	0/42 (0%)	0/38 (0%)	0/31 (0%)	0/8 (0%)
First incidence (days)	–	–	–	–	520
Poly-3 test	P = 0.016*	–	–	–	P = 0.152
All Organs: Fibrous Histiocytoma					
Overall rate	1/47 (2%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	0/48 (0%)
Adjusted rate	2.2%	0.0%	0.0%	2.4%	0.0%
Terminal rate	0/41 (0%)	0/42 (0%)	0/38 (0%)	0/31 (0%)	0/8 (0%)
First incidence (days)	559	–	–	732	–
Poly-3 test	P = 0.549N	P = 0.504N	P = 0.509N	P = 0.737	P = 0.601N
All Organs: Hemangioma					
Overall rate	0/47 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)	0/48 (0%)
Adjusted rate	0.0%	0.0%	0.0%	2.4%	0.0%
Terminal rate	0/41 (0%)	0/42 (0%)	0/38 (0%)	1/31 (3%)	0/8 (0%)
First incidence (days)	–	–	–	733 (T)	–
Poly-3 test	P = 0.357	–	–	P = 0.482	–
All Organs: Hemangiosarcoma					
Overall rate	1/47 (2%)	0/48 (0%)	1/48 (2%)	2/48 (4%)	2/48 (4%)
Adjusted rate	2.2%	0.0%	2.2%	4.8%	7.2%
Terminal rate	1/41 (2%)	0/42 (0%)	0/38 (0%)	1/31 (3%)	0/8 (0%)
First incidence (days)	733 (T)	–	586	621	477
Poly-3 test	P = 0.084	P = 0.501N	P = 0.755	P = 0.471	P = 0.338

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
All Organs: Hemangioma or Hemangiosarcoma					
Overall rate	1/47 (2%)	0/48 (0%)	1/48 (2%)	3/48 (6%)	2/48 (4%)
Adjusted rate	2.2%	0.0%	2.2%	7.2%	7.2%
Terminal rate	1/41 (2%)	0/42 (0%)	0/38 (0%)	2/31 (7%)	0/8 (0%)
First incidence (days)	733 (T)	–	586	621	477
Poly-3 test	P = 0.056	P = 0.501N	P = 0.755	P = 0.276	P = 0.338
All Organs: Histiocytic Sarcoma					
Overall rate	2/47 (4%)	3/48 (6%)	3/48 (6%)	1/48 (2%)	4/48 (8%)
Adjusted rate	4.4%	6.6%	6.7%	2.4%	14.4%
Terminal rate	1/41 (2%)	2/42 (5%)	2/38 (5%)	0/31 (0%)	1/8 (13%)
First incidence (days)	708	698	519	644	639
Poly-3 test	P = 0.202	P = 0.497	P = 0.490	P = 0.530N	P = 0.153
All Organs: Leukemia (Lymphocytic, Monocytic, Mononuclear, or Undifferentiated)					
Overall rate	1/47 (2%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)
Adjusted rate	2.2%	0.0%	0.0%	0.0%	0.0%
Terminal rate	0/41 (0%)	0/42 (0%)	0/38 (0%)	0/31 (0%)	0/8 (0%)
First incidence (days)	683	–	–	–	–
Poly-3 test	P = 0.261N	P = 0.502N	P = 0.508N	P = 0.519N	P = 0.599N
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, NOS, or Undifferentiated Cell Type)					
Overall rate	8/47 (17%)	5/48 (10%)	7/48 (15%)	5/48 (10%)	6/48 (13%)
Adjusted rate	17.5%	11.0%	15.6%	12.0%	20.3%
Terminal rate	8/41 (20%)	5/42 (12%)	5/38 (13%)	5/31 (16%)	2/8 (25%)
First incidence (days)	733 (T)	733 (T)	582	733 (T)	282
Poly-3 test	P = 0.418	P = 0.280N	P = 0.513N	P = 0.338N	P = 0.501
All Organs: Osteosarcoma					
Overall rate	0/47 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	3.6%
Terminal rate	0/41 (0%)	0/42 (0%)	0/38 (0%)	0/31 (0%)	0/8 (0%)
First incidence (days)	–	–	–	–	394
Poly-3 test	P = 0.107	–	–	–	P = 0.406
All Organs: Osteosarcoma or Osteoma					
Overall rate	0/47 (0%)	0/48 (0%)	0/48 (0%)	0/48 (0%)	1/48 (2%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	3.6%
Terminal rate	0/41 (0%)	0/42 (0%)	0/38 (0%)	0/31 (0%)	0/8 (0%)
First incidence (days)	–	–	–	–	394
Poly-3 test	P = 0.107	–	–	–	P = 0.406

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
All Organs: Benign Tumors					
Overall rate	10/47 (21%)	26/48 (54%)	27/48 (56%)	35/48 (73%)	42/48 (88%)
Adjusted rate	21.7%	56.0%	58.8%	75.7%	94.6%
Terminal rate	7/41 (17%)	24/42 (57%)	22/38 (58%)	22/31 (71%)	7/8 (88%)
First incidence (days)	636	537	519	379	384
Poly-3 test	P < 0.001***	P < 0.001***	P < 0.001***	P < 0.001***	P < 0.001***
All Organs: Malignant Tumors					
Overall rate	15/47 (32%)	13/48 (27%)	19/48 (40%)	25/48 (52%)	37/48 (77%)
Adjusted rate	32.2%	28.2%	41.1%	56.7%	87.1%
Terminal rate	11/41 (27%)	10/42 (24%)	12/38 (32%)	14/31 (45%)	6/8 (75%)
First incidence (days)	559	540	519	520	282
Poly-3 test	P < 0.001***	P = 0.425N	P = 0.251	P = 0.014*	P < 0.001***
All Organs: Benign or Malignant Tumors					
Overall rate	23/47 (49%)	35/48 (73%)	34/48 (71%)	41/48 (85%)	44/48 (92%)
Adjusted rate	48.9%	74.0%	71.7%	87.2%	96.2%
Terminal rate	17/41 (42%)	30/42 (71%)	25/38 (66%)	25/31 (81%)	7/8 (88%)
First incidence (days)	559	537	519	379	282
Poly-3 test	P < 0.001***	P = 0.010**	P = 0.018*	P < 0.001***	P < 0.001***

[a]Number of animals with neoplasm per number of animals examined microscopically.
[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c]Observed incidence at the terminal sacrifice.
[d]T indicates terminal sacrifice.
[e]Beneath the 0 mM Glycidamide are the p-values associated with the trend test. Beneath the treated groups incidences are the p-values corresponding to pairwise comparisons between the 0 mM Glycidamide group and the treated groups. The Poly-3 test accounts for differential mortality in animals that do not reach the terminal sacrifice. An N indicates a negative trend or decreased tumor incidence.

Table D-3. Historical Incidence of Alveolar/Bronchiolar Neoplasms in NCTR Control Female B6C3F1/Nctr Mice

Study (Report Date)	Route of Administration	Incidence in Controls		
		Adenoma	Carcinoma	Adenoma or Carcinoma
Sulfamethazine (February 1988)	Diet	5/182 (2.7%)	1/182 (0.5%)	6/182 (3.3%)
Doxylamine (April 1991)	Diet	3/48 (6.3%)	0/48 (0.0%)	3/48 (6.3%)
Tripolidine (June 1991)	Diet	3/47 (6.4%)	2/47 (4.3%)	5/47 (10.6%)
Pyrilamine (July 1991)	Diet	1/48 (2.1%)	0/48 (0.0%)	1/48 (2.1%)
Fumonisin B_1 (March 1999)	Diet	2/47 (4.3%)	0/47 (0.0%)	2/47 (4.3%)
Malachite Green (June 2001)	Diet	2/48 (4.2%)	0/48 (0.0%)	2/48 (4.2%)
Leucomalachite Green (June 2001)	Diet	3/47 (6.4%)	2/47 (4.3%)	5/47 (10.6%)
Chloral Hydrate (July 2001)	Gavage	8/143 (5.6%)	0/143 (0.0%)	8/143 (5.6%)
Urethane and Ethanol (May 2003)	Drinking Water	4/48 (8.3%)	2/48 (4.2%)	6/48 (12.5%)
Acrylamide (February 2012)	Drinking Water	1/47 (2.1%)	1/47 (2.1%)	2/47 (4.3%)
Aloe vera Whole Leaf (2013)	Drinking Water	3/45 (6.7%)	0/45 (0.0%)	3/45 (6.7%)
Total (%) (All studies)		35/750 (4.7%)	8/750 (1.1%)	43/750 (5.7%)
Range		2.1–8.3%	0.0–4.3%	2.1–12.5%
Total (%) (Drinking water studies)		8/140 (5.7%)	3/140 (2.1%)	11/140 (7.9%)
Range		2.1–8.3%	0–4.2%	4.3–12.5%

Table D-4. Historical Incidence of Mammary Gland Neoplasms in NCTR Control Female B6C3F1/Nctr Mice

Study (Report Date)	Route of Administration	Incidence in Controls		
		Adenocarcinoma	Adenoacanthoma	Adenocarcinoma or Adenoacanthoma
Doxylamine (April 1991)	Diet	4/47 (8.5%)	0/47 (0.0%)	4/47 (8.5%)
Tripolidine (June 1991)	Diet	0/44 (0.0%)	0/44 (0.0%)	0/44 (0.0%)
Pyrilamine (July 1991)	Diet	0/47 (0.0%)	0/47 (0.0%)	0/47 (0.0%)
Fumonisin B_1 (March 1999)	Diet	1/46 (2.2%)	0/46 (0.0%)	1/46 (2.2%)
Malachite Green (June 2001)	Diet	2/46 (4.3%)	0/46 (0.0%)	2/46 (4.3%)
Leucomalachite Green (June 2001)	Diet	1/46 (2.2%)	2/46 (4.3%)	3/46 (6.5%)
Chloral Hydrate (July 2001)	Gavage	1/133 (0.8%)	0/133 (0.0%)	1/133 (0.8%)
Urethane and Ethanol (May 2003)	Drinking Water	4/47 (8.5%)	0/47 (0.0%)	4/47 (8.5%)
Acrylamide (February 2012)	Drinking Water	0/47 (0.0%)	0/47 (0.0%)	0/47 (0.0%)
Aloe vera Whole Leaf (2013)	Drinking Water	5/44 (11.4%)	0/44 (0.0%)	5/44 (11.4%)
Total (%) (All studies)		18/547 (3.3%)	2/547 (0.4%)	20/547 (3.7%)
Range		0.0–11.4%	0.0–4.3%	0.0–11.4%
Total (%) (Drinking water studies)		9/138 (6.5%)	0/138 (0.0%)	9/138 (6.5%)
Range		0.0–11.4%	0.0%	0.0–11.4%

Table D-5. Historical Incidence of Adenoma of the Harderian Gland in NCTR Control Female B6C3F1/Nctr Mice

Study (Report Date)	Route of Administration	Incidence in Controls
Sulfamethazine (February 1988)	Diet	13/182 (7.1%)
Doxylamine (April 1991)	Diet	1/48 (2.1%)
Tripolidine (June 1991)	Diet	2/47 (4.3%)
Fumonisin B_1 (March 1999)	Diet	4/46 (8.7%)
Malachite Green (June 2001)	Diet	3/48 (6.3%)
Leucomalachite Green (June 2001)	Diet	2/47 (4.3%)
Chloral Hydrate (July 2001)	Gavage	4/140 (2.9%)
Urethane and Ethanol (May 2003)	Drinking Water	3/48 (6.3%)
Acrylamide (February 2012)	Drinking Water	0/45 (0.0%)
Aloe vera Whole Leaf (2013)	Drinking Water	3/43 (7.0%)
Total (%) (All studies)		35/694 (5.0%)
Range		0.0–8.7%
Total (%) (Drinking water studies)		6/136 (4.4%)
Range		0.0–7.0%

Table D-6. Historical Incidence of Mescenchymal Skin Tumors in NCTR Control Female B6C3F1/Nctr Mice

Study (Report Date)	Route of Administration	Incidence in Controls
Sulfamethazine (February 1988)	Diet	0/181 (0.0%)
Doxylamine (April 1991)	Diet	1/48 (2.1%)
Tripolidine (June 1991)	Diet	0/46 (0.0%)
Pyrilamine (July 1991)	Diet	1/48 (2.1%)
Fumonisin B_1 (March 1999)	Diet	1/47 (2.1%)
Malachite Green (June 2001)	Diet	0/48 (0.0%)
Leucomalachite Green (June 2001)	Diet	0/46 (0.0%)
Chloral Hydrate (July 2001)	Gavage	1/139 (0.7%)
Urethane and Ethanol (May 2003)	Drinking Water	4/48 (8.3%)
Acrylamide (February 2012)	Drinking Water	0/48 (0.0%)
Aloe vera Whole Leaf (2013)	Drinking Water	5/44 (11.4%)
Total (%) (All studies)		13/743 (1.7%)
Range		0.0–11.4%
Total (%) (All studies)		9/140 (6.4%)
Range		0.0–11.4%

Table D-7. Historical Incidence of Squamous Cell Papilloma or Carcinoma (Combined) of the Forestomach in NCTR Control Female B6C3F1/Nctr Mice

Study (Report Date)	Route of Administration	Incidence in Controls
Sulfamethazine (February 1988)	Diet	1/178 (0.6%)
Doxylamine (April 1991)	Diet	0/47 (0.0%)
Tripolidine (June 1991)	Diet	1/46 (2.2%)
Pyrilamine (July 1991)	Diet	1/48 (2.1%)
Fumonisin B_1 (March 1999)	Diet	0/46 (0.0%)
Malachite Green (June 2001)	Diet	2/47 (4.3%)
Leucomalachite Green (June 2001)	Diet	0/46 (0.0%)
Chloral Hydrate (July 2001)	Gavage	1/139 (0.7%)
Urethane and Ethanol (May 2003)	Drinking Water	2/48 (4.2%)
Acrylamide (February 2012)	Drinking Water	4/46 (8.7%)
Aloe vera Whole Leaf (2013)	Drinking Water	0/43 (0.0%)
Total (%) (All studies)		12/734 (1.6%)
Range		0.0–8.7%
Total (%) (Drinking water studies)		6/137 (4.4%)
Range		0.0–8.7%

Table D-8. Historical Incidence of Granulosa Cell Tumors of the Ovary in NCTR Control Female B6C3F1/Nctr Mice

Study (Report Date)	Route of Administration	Incidence in Controls		
		Benign	Malignant	Benign or Malignant
Sulfamethazine (February 1988)	Diet	0/177 (0.0%)	1/177 (0.6%)	1/177 (0.6%)
Doxylamine (April 1991)	Diet	0/47 (0.0%)	0/47 (0.0%)	0/47 (0.0%)
Tripolidine (June 1991)	Diet	0/45 (0.0%)	0/45 (0.0%)	0/45 (0.0%)
Pyrilamine (July 1991)	Diet	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Fumonisin B_1 (March 1999)	Diet	0/46 (0.0%)	0/46 (0.0%)	0/46 (0.0%)
Malachite Green (June 2001)	Diet	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Leucomalachite Green (June 2001)	Diet	0/46 (0.0%)	0/46 (0.0%)	0/46 (0.0%)
Chloral Hydrate (July 2001)	Gavage	1/141 (0.7%)	0/141 (0.0%)	1/141 (0.7%)
Urethane and Ethanol (May 2003)	Drinking Water	0/48 (0.0%)	0/48 (0.0%)	0/48 (0.0%)
Acrylamide (February 2012)	Drinking Water	0/46 (0.0%)	0/46 (0.0%)	0/46 (0.0%)
Aloe vera Whole Leaf (2013)	Drinking Water	0/44 (0.0%)	0/44 (0.0%)	0/44 (0.0%)
Total (%) (All studies)		1/736 (0.1%)	1/736 (0.1%)	2/736 (0.3%)
Range		0.0–0.7%	0.0–0.6%	0.0–0.7%
Total (%) (Drinking water studies)		0/138 (0.0%)	0/138 (0.0%)	0/138 (0.0%)
Range		0.0%	0.0%	0.0%

Table D-9. Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Two-year Drinking Water Study of Glycidamide[a]

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Disposition Summary					
Animals initially in study	48	48	48	48	48
Early deaths					
Moribund sacrifice	2	2	6	7	32
Natural death	1	2	2	4	6
Survivors					
Moribund sacrifice	2	–	2	5	2
Natural death	2	2	–	1	–
Terminal sacrifice	41	42	38	31	8
Animals examined microscopically	47	48	48	48	48
Alimentary System					
Esophagus	(46)	(48)	(46)	(47)	(45)
Gallbladder	(45)	(44)	(47)	(44)	(41)
Infiltration cellular, lymphocyte	–	1 (2%)	–	–	–
Intestine large, cecum	(45)	(44)	(46)	(45)	(42)
Hyperplasia, lymphoid	2 (4%)	–	–	4 (9%)	1 (2%)
Intestine large, colon	(45)	(45)	(47)	(45)	(43)
Intestine large, rectum	(45)	(45)	(46)	(45)	(43)
Intestine small, duodenum	(45)	(44)	(46)	(45)	(42)
Hyperplasia, lymphoid	–	–	–	–	1 (2%)
Intestine small, ileum	(45)	(44)	(46)	(45)	(42)
Hyperplasia, lymphoid	–	–	–	1 (2%)	–
Inflammation, suppurative	–	1 (2%)	–	–	–
Ulcer	–	1 (2%)	–	–	–
Intestine small, jejunum	(45)	(44)	(46)	(45)	(42)
Hyperplasia, lymphoid	–	1 (2%)	1 (2%)	1 (2%)	–
Liver	(47)	(48)	(47)	(46)	(43)
Angiectasis	–	–	1 (2%)	–	5 (12%)
Basophilic focus	2 (4%)	–	3 (6%)	1 (2%)	4 (9%)
Cyst	3 (6%)	–	–	1 (2%)	1 (2%)
Eosinophilic focus	–	2 (4%)	–	–	–
Eosinophilic focus, multiple	–	–	–	–	1 (2%)
Hematopoietic cell proliferation	6 (13%)	11 (23%)	3 (6%)	5 (11%)	6 (14%)
Hemorrhage	1 (2%)	–	–	–	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Infiltration cellular, lymphocyte	4 (9%)	3 (6%)	3 (6%)	4 (9%)	2 (5%)
Inflammation, chronic active	1 (2%)	–	–	5 (11%)	1 (2%)
Necrosis	–	–	–	–	5 (12%)
Tension lipidosis	–	–	–	–	1 (2%)
Vacuolization cytoplasmic	1 (2%)	1 (2%)	1 (2%)	3 (7%)	3 (7%)
Centrilobular, degeneration	–	–	–	–	1 (2%)
Pancreas	(45)	(48)	(47)	(46)	(44)
Infiltration cellular, lymphocyte	9 (20%)	10 (21%)	10 (21%)	9 (20%)	3 (7%)
Necrosis	–	–	–	–	2 (5%)
Acinus, degeneration	2 (4%)	6 (13%)	3 (6%)	–	2 (5%)
Duct, dilatation	–	2 (4%)	–	–	–
Salivary glands	(45)	(47)	(47)	(47)	(44)
Infiltration cellular, lymphocyte	29 (64%)	30 (64%)	28 (60%)	28 (60%)	8 (18%)
Stomach, forestomach	(45)	(45)	(47)	(45)	(44)
Cyst epithelial inclusion	–	1 (2%)	–	–	–
Ulcer	1 (2%)	–	1 (2%)	1 (2%)	1 (2%)
Epithelium, hyperplasia	4 (9%)	4 (9%)	10 (21%)	11 (24%)	5 (11%)
Stomach, glandular	(45)	(45)	(47)	(45)	(43)
Epithelium, hyperplasia	–	–	–	1 (2%)	–
Cardiovascular System					
Blood vessel	(46)	(48)	(47)	(47)	(45)
Heart	(46)	(48)	(47)	(47)	(45)
Cardiomyopathy	–	1 (2%)	–	–	1 (2%)
Inflammation, chronic	–	1 (2%)	–	–	–
Pericardium, inflammation, chronic active	–	–	–	–	1 (2%)
Endocrine System					
Adrenal cortex	(45)	(48)	(47)	(47)	(44)
Accessory adrenal cortical nodule	–	–	–	2 (4%)	–
Angiectasis	–	–	–	1 (2%)	–
Cyst	–	–	–	–	1 (2%)
Hypertrophy	–	1 (2%)	1 (2%)	–	–
Subcapsular, hyperplasia	45 (100%)	48 (100%)	47 (100%)	46 (98%)	43 (98%)
Adrenal medulla	(45)	(45)	(47)	(47)	(43)
Islets, pancreatic	(45)	(48)	(47)	(46)	(43)
Hyperplasia	–	2 (4%)	3 (6%)	2 (4%)	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Parathyroid gland	(41)	(45)	(46)	(40)	(39)
Cyst	4 (10%)	–	2 (4%)	2 (5%)	2 (5%)
Hyperplasia	–	–	–	–	1 (3%)
Inflammation, chronic active	–	–	1 (2%)	–	–
Pituitary gland	(45)	(45)	(43)	(47)	(43)
Pars distalis, cyst	–	1 (2%)	1 (2%)	–	–
Pars distalis, hyperplasia	3 (7%)	–	2 (5%)	1 (2%)	3 (7%)
Thyroid gland	(45)	(47)	(46)	(46)	(44)
Cyst	1 (2%)	1 (2%)	–	–	–
Ectopic thymus	1 (2%)	2 (4%)	3 (7%)	1 (2%)	1 (2%)
Infiltration cellular, lymphocyte	1 (2%)	3 (6%)	–	–	–
Inflammation, chronic active	–	1 (2%)	–	1 (2%)	–
Follicle, degeneration	3 (7%)	5 (11%)	7 (15%)	6 (13%)	3 (7%)
General Body System					
Tissue NOS	(0)	(0)	(0)	(0)	(1)
Genital System					
Clitoral Gland	(44)	(48)	(47)	(47)	(41)
Degeneration	43 (98%)	48 (100%)	47 (100%)	47 (100%)	36 (88%)
Inflammation, suppurative	1 (2%)	–	–	–	–
Inflammation, chronic active	1 (2%)	–	–	–	1 (2%)
Ovary	(45)	(47)	(47)	(46)	(44)
Angiectasis	1 (2%)	–	–	4 (9%)	1 (2%)
Atrophy	44 (98%)	42 (89%)	46 (98%)	39 (85%)	31 (70%)
Cyst	14 (31%)	16 (34%)	23 (49%)	21 (46%)	15 (34%)
Hematocyst	–	–	–	–	1 (2%)
Hemorrhage	1 (2%)	1 (2%)	3 (6%)	2 (4%)	3 (7%)
Hyperplasia, tubulostromal	–	–	–	–	1 (2%)
Inflammation, chronic	1 (2%)	–	–	–	–
Mineralization	1 (2%)	–	–	–	1 (2%)
Thrombosis	1 (2%)	–	1 (2%)	1 (2%)	1 (2%)
Bilateral, cyst	–	1 (2%)	2 (4%)	1 (2%)	3 (7%)
Uterus	(45)	(48)	(47)	(47)	(45)
Angiectasis	1 (2%)	2 (4%)	–	2 (4%)	2 (4%)
Fibrosis	–	1 (2%)	–	–	–
Hemorrhage	–	–	–	1 (2%)	1 (2%)
Hydrometra	1 (2%)	1 (2%)	–	–	–

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Inflammation, suppurative	–	–	1 (2%)	–	–
Necrosis	–	–	–	–	1 (2%)
Thrombus	–	1 (2%)	–	–	2 (4%)
Endometrium, hyperplasia, cystic	44 (98%)	45 (94%)	47 (100%)	45 (96%)	36 (80%)
Endometrium, hyperplasia, glandular, focal	–	–	1 (2%)	–	–
Vagina	(0)	(0)	(0)	(0)	(1)
Hematopoietic System					
Bone marrow	(46)	(47)	(47)	(45)	(43)
Hyperplasia	–	2 (4%)	5 (11%)	6 (13%)	20 (47%)
Lymph node	(8)	(2)	(5)	(8)	(12)
Lumbar, hyperplasia, lymphoid	1 (13%)	–	–	1 (13%)	2 (17%)
Lumbar, infiltration cellular, histiocyte	–	–	–	–	1 (8%)
Lumbar, infiltration cellular, plasma cell	–	–	–	–	1 (8%)
Lumbar, infiltration cellular, polymorphonuclear	–	–	–	1 (13%)	1 (8%)
Mediastinal, angiectasis	–	–	–	–	1 (8%)
Mediastinal, hyperplasia, lymphoid	1 (13%)	–	2 (40%)	–	–
Pancreatic, hyperplasia, lymphoid	–	–	–	1 (13%)	–
Renal, hyperplasia, lymphoid	–	–	–	–	1 (8%)
Renal, infiltration cellular, plasma cell	–	–	–	–	1 (8%)
Renal, infiltration cellular, polymorphonuclear	–	1 (50%)	–	–	1 (8%)
Lymph node, mandibular	(46)	(48)	(47)	(47)	(43)
Erythrophagocytosis	–	–	–	–	1 (2%)
Hemorrhage	1 (2%)	–	–	–	–
Hyperplasia, lymphoid	13 (28%)	10 (21%)	8 (17%)	8 (17%)	–
Infiltration cellular, plasma cell	3 (7%)	1 (2%)	4 (9%)	–	1 (2%)
Infiltration cellular, polymorphonuclear	1 (2%)	2 (4%)	–	–	1 (2%)
Sinus, dilatation	–	–	–	–	1 (2%)
Lymph node, mesenteric	(43)	(46)	(46)	(47)	(43)
Angiectasis	–	2 (4%)	–	2 (4%)	–
Hemorrhage	–	–	–	–	1 (2%)
Hyperplasia, lymphoid	14 (33%)	7 (15%)	9 (20%)	14 (30%)	5 (12%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Infiltration cellular, histiocyte	1 (2%)	–	1 (2%)	2 (4%)	–
Infiltration cellular, mast cell	–	–	1 (2%)	–	–
Infiltration cellular, plasma cell	2 (5%)	1 (2%)	–	–	–
Infiltration cellular, polymorphonuclear	–	1 (2%)	–	–	–
Thrombosis	–	–	–	–	1 (2%)
Sinus, dilatation	1 (2%)	–	–	–	–
Spleen	(46)	(47)	(47)	(47)	(45)
Accessory spleen	–	–	1 (2%)	–	–
Depletion lymphoid	–	–	–	–	1 (2%)
Hematopoietic cell proliferation	6 (13%)	10 (21%)	11 (23%)	14 (30%)	29 (64%)
Hemorrhage	–	–	1 (2%)	–	–
Hyperplasia, lymphoid	35 (76%)	33 (70%)	35 (74%)	29 (62%)	12 (27%)
Necrosis	1 (2%)	–	–	–	–
Pigmentation	1 (2%)	3 (6%)	1 (2%)	1 (2%)	–
Thymus	(43)	(44)	(45)	(43)	(37)
Angiectasis	1 (2%)	–	–	–	–
Atrophy	16 (37%)	11 (25%)	15 (33%)	13 (30%)	18 (49%)
Hyperplasia, lymphoid	7 (16%)	5 (11%)	9 (20%)	5 (12%)	1 (3%)
Mineralization	–	1 (2%)	1 (2%)	–	–
Integumentary System					
Mammary gland	(45)	(48)	(47)	(47)	(45)
Alveolus, hyperplasia	1 (2%)	–	–	2 (4%)	–
Skin	(45)	(48)	(47)	(47)	(45)
Fibrosis	–	–	–	2 (4%)	–
Inflammation, chronic active	–	–	–	1 (2%)	–
Musculoskeletal System					
Bone	(0)	(0)	(1)	(2)	(1)
Joint, ligament, degeneration	–	–	1 (100%)	–	–
Bone, femur	(46)	(48)	(48)	(48)	(48)
Callus	–	–	–	–	1 (2%)
Fibro-osseous lesion	1 (2%)	–	–	1 (2%)	–
Skeletal muscle	(46)	(48)	(47)	(47)	(45)
Atrophy	–	–	–	–	1 (2%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Nervous System					
Brain, brain stem	(45)	(47)	(47)	(45)	(44)
Brain, cerebellum	(45)	(47)	(47)	(45)	(44)
Hemorrhage	–	–	–	–	1 (2%)
Infiltration cellular, lymphocyte	–	1 (2%)	–	–	1 (2%)
Brain, cerebrum	(45)	(47)	(47)	(45)	(44)
Compression	–	–	–	1 (2%)	
Hemorrhage	–	–	–	–	1 (2%)
Infarct, focal, chronic	–	–	–	–	1 (2%)
Infiltration cellular, lymphocyte	3 (7%)	1 (2%)	–	–	–
Mineralization	31 (69%)	36 (77%)	28 (60%)	25 (56%)	22 (50%)
Hippocampus, neuron, depletion	–	–	1 (2%)	–	–
Peripheral nerve, sciatic	(45)	(47)	(47)	(45)	(44)
Inflammation, acute	–	–	1 (2%)	–	–
Axon, degeneration	39 (87%)	38 (81%)	38 (81%)	34 (76%)	28 (64%)
Spinal cord, cervical	(45)	(44)	(47)	(45)	(43)
Infiltration cellular, lymphocyte	3 (7%)	–	1 (2%)	1 (2%)	–
Axon, degeneration	4 (9%)	9 (20%)	10 (21%)	9 (20%)	10 (23%)
Nerve, degeneration	–	–	–	–	1 (2%)
Spinal cord, lumbar	(45)	(45)	(47)	(45)	(43)
Cyst	–	–	1 (2%)	–	–
Infiltration cellular, lymphocyte	6 (13%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Axon, degeneration	12 (27%)	13 (29%)	13 (28%)	15 (33%)	4 (9%)
Nerve, degeneration	43 (96%)	43 (96%)	40 (85%)	40 (89%)	24 (56%)
Spinal cord, thoracic	(45)	(44)	(47)	(45)	(43)
Infiltration cellular, lymphocyte	3 (7%)	–	1 (2%)	1 (2%)	–
Axon, degeneration	35 (78%)	32 (73%)	34 (72%)	34 (76%)	23 (53%)
Nerve, degeneration	–	1 (2%)	–	1 (2%)	–
Respiratory System					
Lung	(46)	(48)	(47)	(47)	(44)
Congestion	–	1 (2%)	–	–	–
Hemorrhage	2 (4%)	–	–	–	1 (2%)
Infiltration cellular, histiocyte	–	–	1 (2%)	2 (4%)	3 (7%)
Infiltration cellular, lymphocyte	1 (2%)	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Inflammation, chronic active	3 (7%)	2 (4%)	1 (2%)	–	–
Alveolar epithelium, hyperplasia	–	1 (2%)	2 (4%)	4 (9%)	3 (7%)

	0 mM	0.0875 mM	0.175 mM	0.35 mM	0.70 mM
Nose	(46)	(46)	(47)	(46)	(45)
Amyloid deposition	–	–	1 (2%)	–	–
Crystals	–	–	2 (4%)	–	1 (2%)
Hyaline droplet	9 (20%)	3 (7%)	15 (32%)	4 (9%)	–
Inflammation, suppurative	–	1 (2%)	–	–	1 (2%)
Trachea	(45)	(47)	(46)	(46)	(43)
Atrophy	–	–	–	1 (2%)	–
Special Senses System					
Eye	(45)	(44)	(47)	(44)	(43)
Cataract	1 (2%)	2 (5%)	8 (17%)	8 (18%)	9 (21%)
Inflammation, chronic active	–	1 (2%)	–	–	–
Phthisis bulbi	–	1 (2%)	–	1 (2%)	2 (5%)
Cornea, fibrosis	–	–	–	–	1 (2%)
Cornea, inflammation, chronic active	–	2 (5%)	1 (2%)	3 (7%)	5 (12%)
Cornea, ulcer	–	–	–	–	1 (2%)
Harderian gland	(45)	(47)	(47)	(46)	(46)
Degeneration, cystic	–	–	–	1 (2%)	–
Fibrosis	–	–	1 (2%)	1 (2%)	–
Infiltration cellular, lymphocyte	3 (7%)	5 (11%)	1 (2%)	2 (4%)	–
Epithelium, hyperplasia	–	1 (2%)	–	1 (2%)	–
Urinary System					
Kidney	(46)	(47)	(47)	(46)	(43)
Cyst	–	–	–	–	1 (2%)
Hyaline droplet	–	1 (2%)	1 (2%)	–	3 (7%)
Hydronephrosis	–	–	1 (2%)	–	1 (2%)
Infarct	–	–	1 (2%)	–	–
Infiltration cellular, lymphocyte	23 (50%)	26 (55%)	28 (60%)	19 (41%)	13 (30%)
Inflammation, chronic	–	–	1 (2%)	–	–
Metaplasia, osseous	2 (4%)	1 (2%)	–	–	–
Nephropathy	2 (4%)	2 (4%)	3 (6%)	5 (11%)	5 (12%)
Glomerulus, amyloid deposition	1 (2%)	–	–	1 (2%)	–
Urinary bladder	(45)	(46)	(47)	(46)	(42)
Infiltration cellular, lymphocyte	31 (69%)	28 (61%)	34 (72%)	22 (48%)	18 (43%)
Lumen, dilatation	–	–	–	2 (4%)	1 (2%)

[a]Number of animals examined microscopically at the site and the number of animals with lesion.

Appendix E. Organ Weights and Organ-Weight-to-Body-Weight Ratios

Tables

Table E-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Two-week Drinking Water Study of Glycidamide[a]

	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM	7.03 mM
Male							
Necropsy body wt.	133.6 ± 6.7	137.2 ± 7.8	126.8 ± 4.8	137.5 ± 7.3	134.9 ± 4.4	111.4 ± 1.7*	79.2 ± 0.8*
Brain							
Absolute	1.68 ± 0.05	1.68 ± 0.05	1.60 ± 0.05	1.67 ± 0.03	1.61 ± 0.03	1.52 ± 0.04	1.43 ± 0.02*
Liver							
Absolute	6.59 ± 0.35	7.08 ± 0.33	6.03 ± 0.39	6.87 ± 0.50	7.31 ± 0.22	5.73 ± 0.19	4.06 ± 0.16*
Relative[c]	4.94 ± 0.13	5.17 ± 0.08	4.74 ± 0.17	4.98 ± 0.11	5.42 ± 0.07	5.14 ± 0.12	5.12 ± 0.20
Relative[d]	3.93 ± 0.16	4.22 ± 0.21	3.77 ± 0.23	4.10 ± 0.23	4.55 ± 0.19	3.78 ± 0.14	2.85 ± 0.11*
Female							
Necropsy body wt.	96.9 ± 4.2	96.3 ± 9.3	99.7 ± 10.3	99.8 ± 4.5	97.4 ± 2.8	83.0 ± 1.9	55.7 ± 9.9[b,*]
Brain							
Absolute	1.56 ± 0.02	1.58 ± 0.06	1.52 ± 0.08	1.54 ± 0.04	1.55 ± 0.03	1.44 ± 0.02	1.36 ± 0.03[b,*]
Liver							
Absolute	4.95 ± 0.31	4.66 ± 0.44	4.63 ± 0.32	4.73 ± 0.24	4.79 ± 0.15	3.85 ± 0.05	2.61 ± 0.51[b,*]
Relative[c]	5.10 ± 0.16	4.85 ± 0.11	4.70 ± 0.21	4.74 ± 0.07	4.92 ± 0.07	4.65 ± 0.17	4.66 ± 0.09[b]
Relative[d]	3.18 ± 0.16	2.94 ± 0.20	3.04 ± 0.10	3.09 ± 0.19	3.08 ± 0.10	2.68 ± 0.04	1.92 ± 0.35[b,*]

[a]The data are presented in grams as the mean ± s.e.m. An asterisk (*) denotes significant difference ($p < 0.05$) from the control.
[b]Data based upon three rats only.
[c]Liver weight/body weight × 100.
[d]Liver weight/brain weight.

Table E-2. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Three-month Drinking Water Study of Glycidamide[a]

	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM
Male						
Necropsy body wt.	325.2 ± 8.6	314.0 ± 5.0	325.4 ± 7.0	310.0 ± 3.6	279.8 ± 7.0*	253.5 ± 4.2*
Brain						
Absolute	1.96 ± 0.04	1.93 ± 0.03	1.99 ± 0.02	1.96 ± 0.02	1.87 ± 0.03	1.77 ± 0.03*
Liver						
Absolute	9.81 ± 0.37	8.79 ± 0.20	9.47 ± 0.40	9.41 ± 0.17	8.09 ± 0.38*	7.93 ± 0.29*
Relative[b]	3.03 ± 0.12	2.80 ± 0.04	2.91 ± 0.10	3.04 ± 0.05	2.89 ± 0.08	3.13 ± 0.11
Relative[c]	5.01 ± 0.18	4.55 ± 0.05	4.76 ± 0.21	4.82 ± 0.09	4.33 ± 0.19*	4.48 ± 0.17
Female						
Necropsy body wt.	207.6 ± 12.6	204.7 ± 16.7	178.2 ± 5.3	190.9 ± 15.6	162.8 ± 5.5	166.0 ± 14.2
Brain						
Absolute	1.82 ± 0.02	1.77 ± 0.02	1.76 ± 0.03	1.71 ± 0.03*	1.70 ± 0.02*	1.59 ± 0.03*

	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM
Liver						
Absolute	5.39 ± 0.18	4.80 ± 0.15*	4.51 ± 0.16*	4.73 ± 0.17*	4.32 ± 0.16*	4.17 ± 0.10*
Relative[b]	2.66 ± 0.17	2.42 ± 0.14	2.54 ± 0.10	2.61 ± 0.24	2.69 ± 0.18	2.61 ± 0.17
Relative[c]	2.96 ± 0.10	2.73 ± 0.11	2.57 ± 0.08*	2.77 ± 0.11	2.55 ± 0.10*	2.63 ± 0.09

[a]The data are presented in grams as the mean ± s.e.m. An asterisk (*) denotes significant difference ($p < 0.05$) from the control.
[b]Liver weight/body weight × 100.
[c]Liver weight/brain weight.

Table E-3. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Two-week Drinking Water Study of Glycidamide

	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM	7.03 mM
Male							
Necropsy body wt.[a]	20.7 ± 1.0	19.4 ± 0.6	21.4 ± 0.6	19.7 ± 0.8[b]	19.6 ± 0.4	18.0 ± 0.7*	13.0 ± 0.6*
Brain							
Absolute[c]	432 ± 9	435 ± 11	426 ± 22	424 ± 13[b]	415 ± 12	422 ± 14	396 ± 6
Liver							
Absolute	902 ± 38	868 ± 27	969 ± 42	840 ± 83[b]	817 ± 41	743 ± 26	577 ± 32*
Relative[d]	4.38 ± 0.12	4.48 ± 0.08	4.53 ± 0.12	4.24 ± 0.24[b]	4.16 ± 0.14	4.15 ± 0.11	4.43 ± 0.13
Relative[e]	2.09 ± 0.07	2.00 ± 0.04	2.28 ± 0.07	1.99 ± 0.23[b]	1.97 ± 0.05	1.77 ± 0.08	1.46 ± 0.09*
Female							
Necropsy body wt.	15.5 ± 0.2	16.0 ± 0.07	15.8 ± 0.3	16.1 ± 0.3	15.8 ± 0.2	14.6 ± 0.6	11.4 ± 0.4*
Brain							
Absolute	433 ± 6	422 ± 13	425 ± 6	432 ± 11	417 ± 8	411 ± 5	377 ± 6*
Liver							
Absolute	629 ± 9	652 ± 11	650 ± 6	684 ± 19	650 ± 24	601 ± 30	507 ± 36*
Relative[d]	4.06 ± 0.10	4.08 ± 0.09	4.13 ± 0.07	4.25 ± 0.19	4.12 ± 0.11	4.15 ± 0.24	4.43 ± 0.24
Relative[e]	1.45 ± 0.03	1.55 ± 0.04	1.53 ± 0.03	1.59 ± 0.07	1.56 ± 0.05	1.46 ± 0.07	1.35 ± 0.08

[a]Body weight data are presented in grams as the mean ± s.e.m. An asterisk (*) denotes significant difference ($p < 0.05$) from the control.
[b]Data based upon three mice only.
[c]Organ weight data are presented in milligrams as the mean ± s.e.m. An asterisk (*) denotes significant difference ($p < 0.05$) from the control.
[d]Liver weight/body weight × 0.1.
[e]Liver weight/brain weight.

Table E-4. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Drinking Water Study of Glycidamide[a]

	0 mM	0.14 mM	0.35 mM	0.70 mM	1.41 mM	3.52 mM
Male						
Necropsy body wt.	25.6 ± 0.5	25.7 ± 0.3	27.5 ± 0.8*	24.4 ± 0.3	24.4 ± 0.6	22.7 ± 0.2*
Brain						
Absolute	0.46 ± 0.009	0.47 ± 0.009	0.45 ± 0.005	0.46 ± 0.007	0.44 ± 0.005	0.43 ± 0.005*
Liver						
Absolute	1.07 ± 0.04	1.07 ± 0.02	1.20 ± 0.06	1.04 ± 0.01	1.02 ± 0.03	0.96 ± 0.02
Relative[b]	4.17 ± 0.08	4.16 ± 0.06	4.33 ± 0.11	4.28 ± 0.05	4.16 ± 0.06	4.24 ± 0.09
Relative[c]	2.31 ± 0.10	2.26 ± 0.07	2.63 ± 0.13*	2.28 ± 0.05	2.31 ± 0.08	2.24 ± 0.05
Female						
Necropsy body wt.	21.3 ± 0.3	20.7 ± 0.4	21.7 ± 0.7	22.4 ± 1.4	21.4 ± 0.3	18.6 ± 0.3*
Brain						
Absolute	0.47 ± 0.009	0.45 ± 0.01	0.47 ± 0.01	0.47 ± 0.007	0.46 ± 0.009	0.44 ± 0.005
Liver						
Absolute	0.91 ± 0.01	0.93 ± 0.02	0.91 ± 0.03	0.91 ± 0.01	0.98 ± 0.02	0.83 ± 0.03
Relative[b]	4.27 ± 0.09	4.51 ± 0.10	4.21 ± 0.13	4.14 ± 0.23	4.58 ± 0.07	4.49 ± 0.10
Relative[c]	1.94 ± 0.05	2.06 ± 0.06	1.95 ± 0.05	1.94 ± 0.05	2.14 ± 0.06	1.90 ± 0.07

[a]The data are presented in grams as the mean ± s.e.m. An asterisk (*) denotes significant difference ($p < 0.05$) from the control.
[b]Liver weight/body weight × 100.
[c]Liver weight/brain weight.

Appendix F. Chemical Characterization and Dose Formulation Studies

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F.1. Procurement and Characterization of Glycidamide

The glycidamide test article used was purchased from Toronto Research Chemicals, Inc. of North York, Ontario Canada (Lot 4-AQL-43-5) and received July 7, 2003. The compound was stored under argon at −20°C. Identity, purity, and stability analyses were conducted by the Division of Biochemical Toxicology Chemistry Support Group (DBT/CHEM) at the National Center for Toxicological Research (NCTR; Jefferson, AR). Reports on analyses performed in support of the glycidamide studies are on file at NCTR.

Test sample characterization was performed using gas chromatography with electron impact mass spectrometry (GC/EI-MS; MS model TSQ 700; DB-5 capillary GC column). The sample (1.5 mg) was dissolved with 1 ml of methanol and diluted 10-fold with methanol. GC/MS analyses were performed with the SPI Injector held at 200°C. A sample of acrylamide from previous analyses was analyzed to compare retention times and purity. Only one major component was apparent in the sample, with a purity >99%. Additional test sample characterization was performed using liquid chromatography with electrospray ionization mass spectrometry (LC/+ESI-MS; MS model TSQ 7000; Aquasil C18 column). The sample was dissolved in methanol to form a 1.5 mg/ml solution, which was diluted 10-fold with mobile phase. An initial gradient analysis with acetonitrile was performed to determine that no noticeable impurities were present. Subsequent analyses were performed isocratic, with H_2O + 0.1% formic acid. Based on the mass spectra and product ion spectra obtained (t_r = 2.5 min; m/z 88), the major component was tentatively identified as glycidamide (CAS# 5694-00-8). No impurities were found with retention times greater than that of the major component.

Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra of the glycidamide samples (Lot 4-AQL-43-5) were recorded with deuterated methanol as solvent (40 mg/ml for ^{1}H NMR and 173 mg/ml for ^{13}C NMR). The samples were briefly bubbled with argon as a precautionary measure and ^{1}H NMR spectra were recorded as a function of time to check for instability. The ^{1}H and ^{13}C NMR spectral parameters were consistent with the glycidamide structure (Figure F-1 and Figure F-2). Additionally, two small broad proton resonances (7.48 and 7.22 ppm) from the NH_2 group were detected in the vertically expanded ^{1}H NMR spectrum. These arise from residual exchangeable protons that were present even though deuterated methanol was used as solvent. The ^{1}H NMR spectrum exhibited considerable impurity. Perhaps most noteworthy is a relatively large singlet at 5.48 ppm, which is consistent with methylene chloride at a level of 0.13% (g/g). The possibility that the brief bubbling with argon lowered the amount of this material cannot be ruled out. There also appears to be a singlet at 54.80 ppm in the ^{13}C NMR spectrum, which would likewise be consistent with methylene chloride, however, the signal is near the limit of detection due to the lower sensitivity of ^{13}C NMR. The largest impurity resonances were assigned to acrylamide at 0.7% (mole/mole). These were detected in both the ^{1}H and ^{13}C NMR spectra. Other impurity resonances, all in the aliphatic region, suggest additional impurities totaling roughly 0.6% (g/g). A very rough estimate of the total organic impurity is 1.5% (g/g).

Purity analysis of samples of glycidamide Toronto Research Chemicals Lot # 4-AQL-43-5 was determined by capillary gas chromatography with flame ionization detection (GC/FID). The GC instrument was a Hewlett Packard HP 6980A operated in the capillary split mode (1:10) using a 320 μm diameter, 30 m length, 0.25 μm film thickness fused silica capillary J&W DB-1701, and

the oven programmed from 35°C (0.2 min hold) to 200°C (2 min final hold). The capillary inlet and FID detector temperatures were held at 200°C and 250°C, respectively. One microliter injections of 2 mg/ml solutions of the test compound in EtOAc were made and compared to EtOAc solvent blank using GC/FID analysis. Glycidamide was analyzed for purity based on the percentage of total area observed by GC/FID response. Three impurity peaks were evident at 7.8, 10.4, and 10.6 min retention, indicating respective contents of 0.3, 1.1, and 0.2% for a total of 1.6% impurities (Figure F-3). Therefore, based on GC/FID, the purity of the glycidamide was 98.4%. Glycidamide samples (Lot 4-AQL-43-5) were analyzed for purity at approximately 1, 1.5, and 2 years into the 2-year study. Each evaluation including the end-of-study analysis indicated that no change had occurred in Lot 4-AQL-43-5 during the course of study.

F.2. Preparation and Analysis of Dose Formulations

The dose formulations were prepared by dissolving glycidamide in water to give the required concentrations (Table F-1). The dose formulations were mixed for 5 min. in high density polyethylene plastic drums (Saint-Gobain Performance Plastics) by a Leeson paddle-stirrer with a four inch stainless steel impeller and stored in stainless steel cases with lids at 4°C.

Periodic analyses of the dose formulations of glycidamide were conducted using GC-FID. Dose formulations were analyzed twice during the 2-week studies (Table F-2), approximately every 2 weeks during the 3-month studies (Table F-3), and approximately every 2 to 3 months during the 2-year studies (Table F-4). Of the dose formulations analyzed and used during the 2-week studies, 11 of 12 were within 10% of the target concentrations. Of the dose formulations analyzed and used during the 3-month studies, 25 of 35 were within 10% of the target concentrations. Of the dose formulations analyzed and used during the 2-year studies, 51 of 52 were within 10% of the target concentrations.

Table F-1. Preparation and Storage of Dose Formulations in the Drinking Water Study of Glycidamide

Two-week Studies	Three-month Studies	Two-year Studies
Preparation		
A stock solution of glycidamide (6.12 mg/ml) in water was prepared in a volumetric flask. The stock solution was diluted with Millipore filtered water to the needed concentrations. The dose formulations in drinking water were prepared weekly.	Same as 2-week studies	Same as 2-week studies
Chemical Lot Number		
4-AQL-43-5	Same as 2-week studies	Same as 2-week studies
Storage Conditions		
Dosed water was stored at 4°C with protection from light.	Same as 2-week studies	Same as 2-week studies
Study Laboratory		
National Center for Toxicological Research (Jefferson, Arkansas)	Same as 2-week studies	Same as 2-week studies

Table F-2. Results of Analyses of Dose Formulations Administered to Rats and Mice in the Two-week Drinking Water Study of Glycidamide

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
April 12, 2004	July 19, 2004	0	<LOQ[b]	–
		12.2	10.4	−15
		30.6	33.4	+9
		61.2	61.5	+1
		122	119	−2
		306	337	+10
		612	648	+6
April 19, 2004	July 19, 2004	0	<LOQ	–
		12.2	11.4	−7
		30.6	30.1	−2
		61.2	66.7	+9
		122	126	+3
		306	291	−5
		612	652	+7

[a]Dosed water was analyzed in duplicate and the average is reported.
[b]Limit of quantitation determined by GC-FID was 2 ppm.

Table F-3. Results of Analyses of Dose Formulations Administered to Rats and Mice in the Three-month Drinking Water Study of Glycidamide

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
July 28, 2004	August 19, 2004	0	<LOQ[b]	–
		12.2	9.1[c]	−25
		30.6	22.7[d]	−26
		61.2	49.0[d]	−20
		122	98.1[d]	−20
		306	258[d]	−16
August 16, 2004	August 27, 2004	0	<LOQ	–
		12.2	12.6[e]	+4
		30.6	22.3[d]	−27
		61.2	55.7	−9
		122	113	−7
		306	229[d]	−25
August 24, 2004	August 26, 2004	0	<LOQ	–
		12.2	11.4	−7
		30.6	29.8	−3
		61.2	65.1	+6
		122	124	+2
		306	303	−1
September 10, 2004	October 5, 2004	0	<LOQ	–
		12.2	10.6	−13
		30.6	29.9	−2
		61.2	70.9[d]	+16
		122	111	−9
		306	305	0
September 24, 2004	October 5, 2004	0	<LOQ	–
		12.2	10.6	−13
		30.6	31.0	+1
		61.2	59.2	−3
		122	133	+9
		306	333	+9
October 8, 2004	October 15, 2004	0	<LOQ	–
		12.2	12.5	+3
		30.6	31.0	+1

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
		61.2	64.5	+6
		122	133	+9
		306	329	+7
October 21, 2004	November 1, 2004	0	<LOQ	–
		12.2	12.9	+6
		30.6	28.2	−8
		61.2	57.8	−5
		122	115	−5
		306	293	−4

[a]Dosed water was analyzed in duplicate and the average is reported.
[b]Limit of quantitation determined by GC-FID was 5 µg/ml.
[c]Based on analysis of n = 3.
[d]Based on analysis of n = 4.
[e]Based on a single sample analysis.

Table F-4. Results of Analyses of Dose Formulations Administered to Rats and Mice in the Two-year Drinking Water Studies of Glycidamide

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
May 24, 2005	June 1, 2005	0	<LOQ[b]	–
		7.65	7.31 ± 1.00	−3
		15.3	15.6 ± 0.4	+2
		30.6	29.8 ± 1.1	−3
		61.2	66.3 ± 0.2	+8
August 2, 2005	August 5, 2005	0	<LOQ	–
		7.65	7.44 ± 0.10	−3
		15.3	15.4 ± 0.4	+1
		30.6	31.8 ± 0.2	+4
		61.2	64.6 ± 4.8	+5
October 11, 2005	October 14, 2005	0	<LOQ	–
		7.65	7.29 ± 0.32	−5
		15.3	16.1 ± 1.2	+5
		30.6	31.4 ± 1.3	+3
		61.2	63.6 ± 4.7	+4
December 13, 2005	December 15, 2005	0	<LOQ	–
		7.65	8.11 ± 0.63	+6
		15.3	14.4 ± 0.2	−6

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
		30.6	30.7 ± 1.4	0
		61.2	63.9 ± 5.5	+4
February 14, 2006	February 15, 2006	0	<LOQ	–
		7.65	8.42 ± 0.30	+10
		15.3	15.4 ± 0.3	+1
		30.6	29.5 ± 1.7	−4
		61.2	63.4 ± 0.2	+4
April 25, 2006	May 1, 2006	0	<LOQ	–
		7.65	7.85 ± 0.15	+3
		15.3	13.8 ± 1.4	−10
		30.6	28.2 ± 0.1	−8
		61.2	65.9 ± 4.9	+8
July 4, 2006	July 11, 2006	0	<LOQ	–
		7.65	7.96[c]	+4
		15.3	15.1 ± 0.5	−1
		30.6	28.2 ± 0.6	−8
		61.2	63.7 ± 8.6	+4
September 5, 2006	September 18, 2006	0	<LOQ	–
		7.65	7.69 ± 0.21	+1
		15.3	16.5 ± 0.6	+8
		30.6	33.7 ± 1.2	+10
		61.2	59.3 ± 7.8	−3
November 14, 2006	November 20, 2006	0	<LOQ	–
		7.65	7.42 ± 0.65	+1
		15.3	14.7 ± 2.0	+8
		30.6	29.5 ± 5.1	+10
		61.2	57.0 ± 11.9	−7
January 23, 2007	January 29, 2007	0	<LOQ	–
		7.65	7.61 ± 1.5	0
		15.3	15.7 ± 1.4	+3
		30.6	32.2 ± 2.4	+5
		61.2	61.0 ± 13.0	0
March 27, 2007	March 29, 2007	0	<LOQ	–
		7.65	7.57 ± 1.78	−1
		15.3	15.4 ± 0.5	+1

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
		30.6	30.5 ± 2.3	0
		61.2	60.4 ± 0.1	−1
June 5, 2007	June 11, 2007	0	<LOQ	–
		7.65	8.36 ± 0.16	+9
		15.3	16.1 ± 0.5	+5
		30.6	29.5 ± 5.9	−4
		61.2	56.4 ± 12.5	−8
August 14, 2007	August 17, 2007	0	<LOQ	–
		7.65	6.54 ± 0.23	−14
		15.3	15.1 ± 1.2	−1
		30.6	29.9 ± 0.3	−2
		61.2	58.7 ± 3.2	−4

[a]Dosed water was analyzed in duplicate and the average ± S.D. is reported.
[b]Limit of quantitation determined by GC-FID was 1.5 µg/ml.
[c]Based on a single sample analysis.

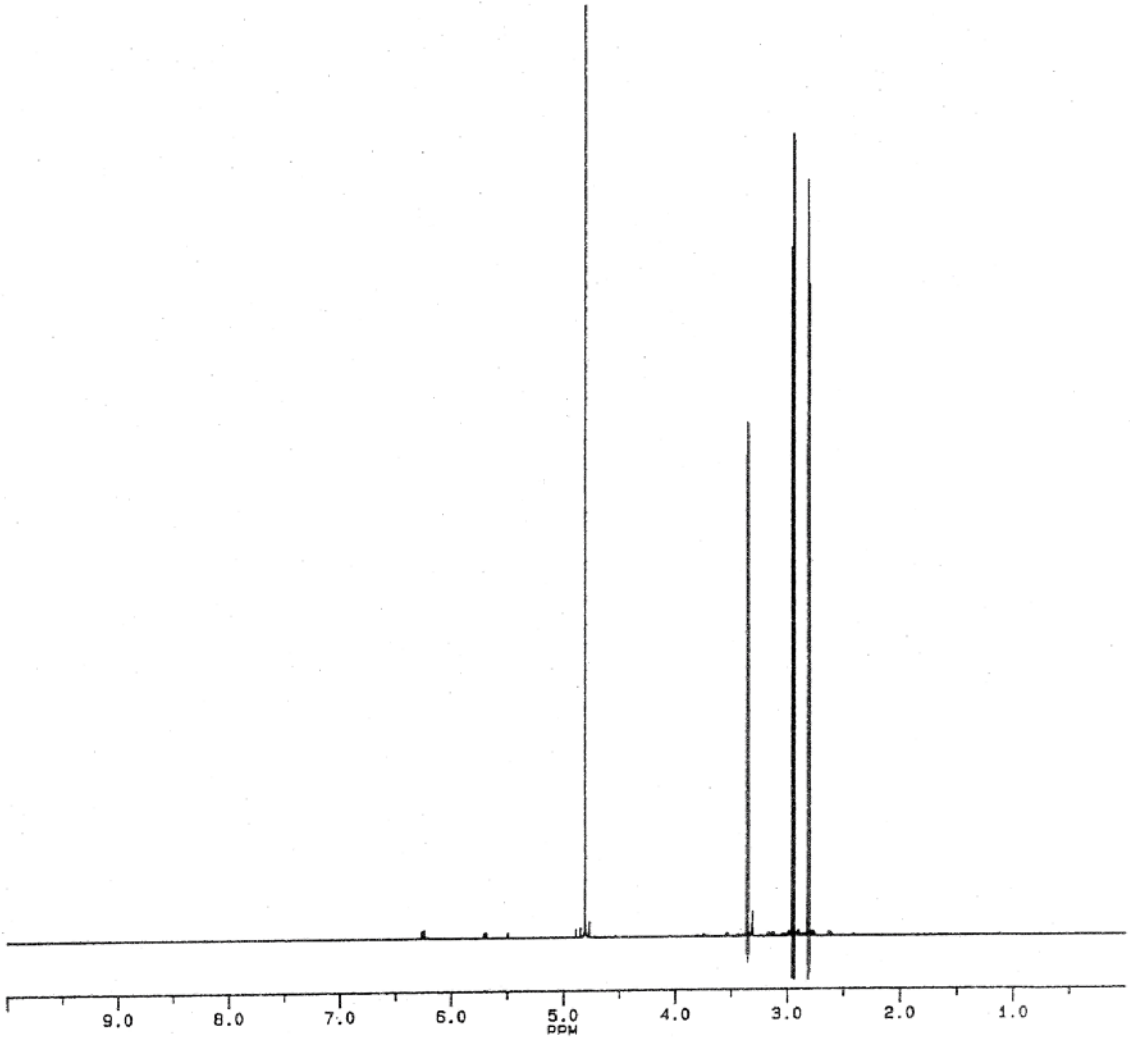

Figure F-1. Proton Nuclear Magnetic Resonance Spectrum of Glycidamide

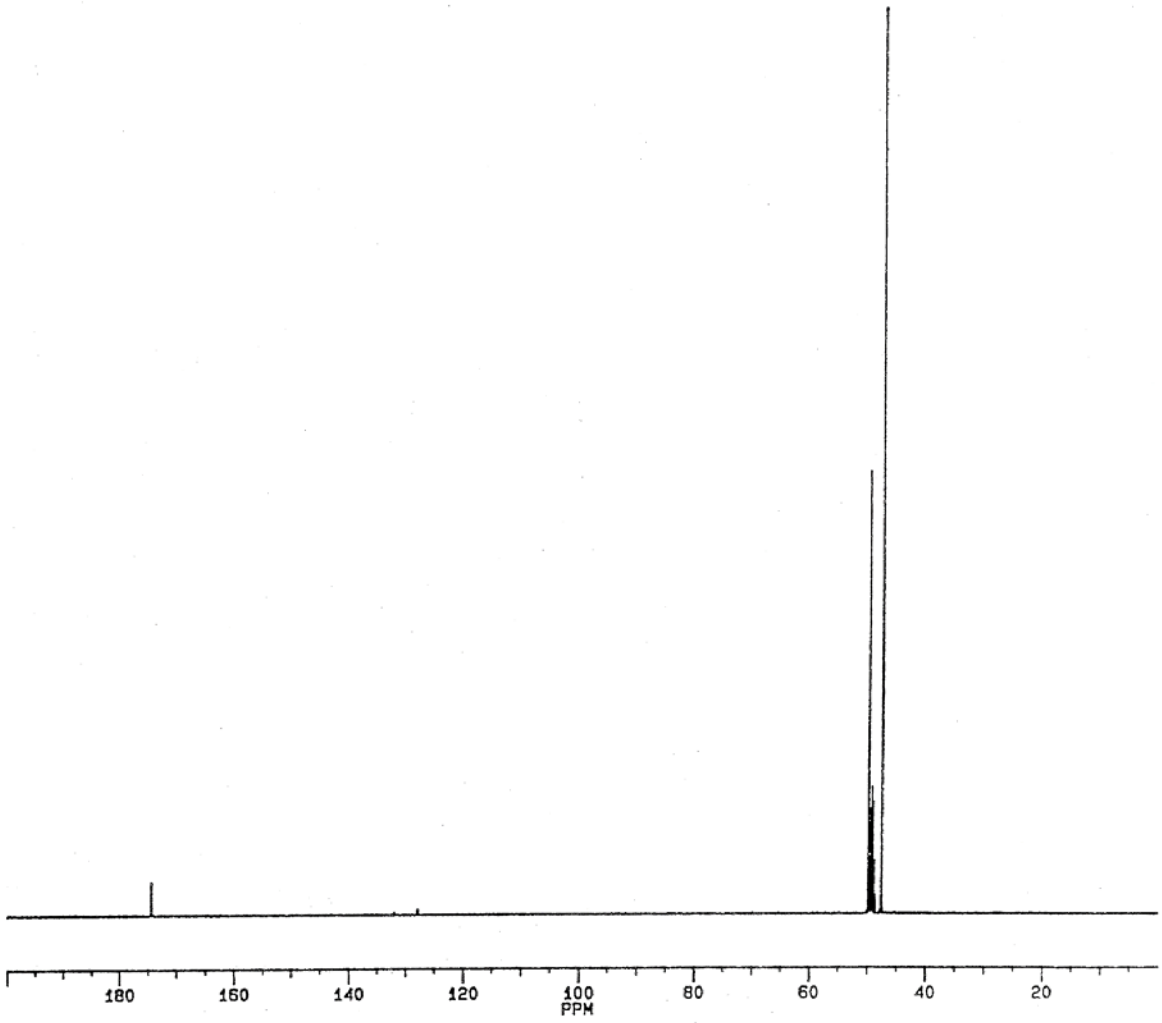

Figure F-2. Carbon Nuclear Magnetic Resonance Spectrum of Glycidamide

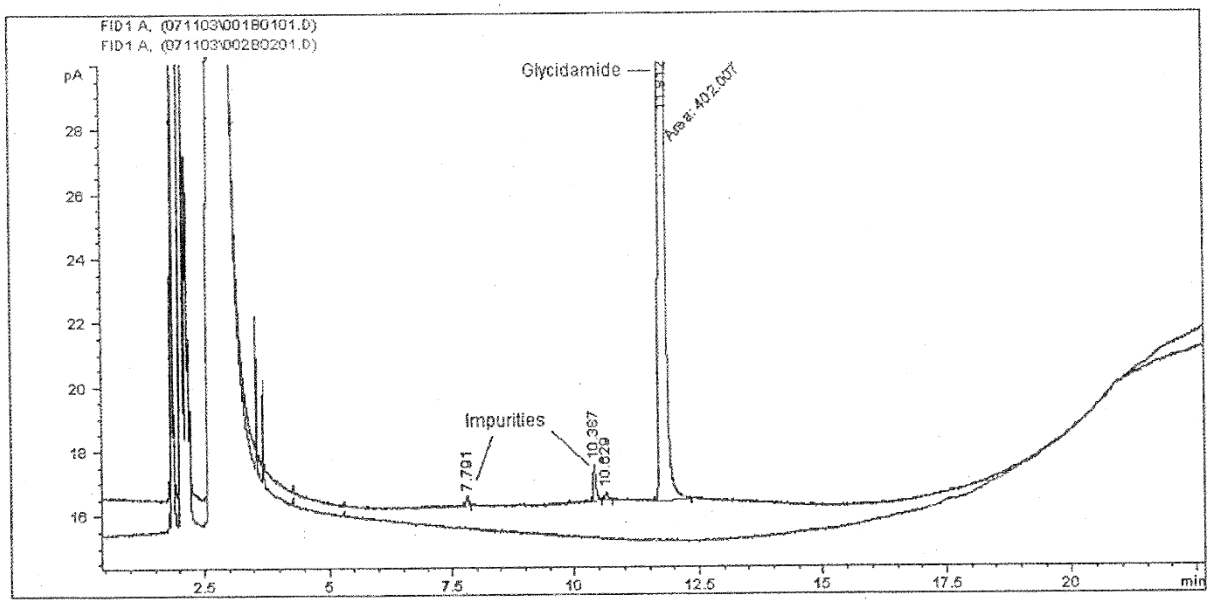

Figure F-3. Capillary Gas Chromatography with Flame Ionization Detection Purity Analysis of Glycidamide

Appendix G. Food Consumption in the Two-year Drinking Water Study of Glycidamide

Tables

Table G-1. Food Consumption of Male Rats in the Two-year Drinking Water Study of Glycidamide

Week[a]	0.0 mM			0.0875 mM				0.175 mM				0.35 mM				0.70 mM			
	N[b]	Mean ± SE[c]	P-Value[d]	N	Mean ± SE	Pct[e]	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value
4	24	16.4 ± 0.2	0.387	24	16.5 ± 0.2	100.5	0.995	24	16.3 ± 0.2	99.6	0.997	24	16.1 ± 0.2	98.3	0.724	24	16.2 ± 0.2	99.1	0.962
8	24	16.4 ± 0.2	0.246	24	16.4 ± 0.2	100.1	1.000	24	16.3 ± 0.2	99.3	0.975	24	16.3 ± 0.2	99.7	0.999	24	16.1 ± 0.2	98.3	0.672
12	24	16.9 ± 0.2	0.049	24	16.1 ± 0.2	95.8	0.047	24	16.3 ± 0.2	97.0	0.234	24	16.1 ± 0.2	95.8	0.051	24	16.1 ± 0.2	95.5	0.032
16	24	16.5 ± 0.2	0.403	24	16.1 ± 0.2	97.7	0.290	24	16.2 ± 0.2	98.0	0.419	24	16.0 ± 0.2	97.1	0.125	24	16.2 ± 0.2	98.2	0.515
20	24	16.6 ± 0.2	0.531	24	16.6 ± 0.2	99.8	1.000	24	16.7 ± 0.2	100.3	0.999	24	16.4 ± 0.2	98.8	0.834	24	16.5 ± 0.2	99.4	0.983
24	24	16.9 ± 0.2	0.530	24	16.9 ± 0.2	100.5	0.997	24	16.7 ± 0.2	99.1	0.955	24	16.9 ± 0.2	100.5	0.997	24	17.0 ± 0.2	100.9	0.964
28	24	17.1 ± 0.2	0.769	24	17.1 ± 0.2	99.7	0.999	24	16.7 ± 0.2	97.8	0.392	24	16.9 ± 0.2	98.8	0.863	24	17.0 ± 0.2	99.3	0.968
32	24	17.1 ± 0.2	0.728	24	17.1 ± 0.2	99.9	1.000	24	17.0 ± 0.2	99.3	0.982	24	17.0 ± 0.2	99.7	0.999	24	17.1 ± 0.2	100.4	0.997
36	24	16.9 ± 0.2	0.974	24	17.0 ± 0.2	100.5	0.994	24	16.9 ± 0.2	99.5	0.993	24	16.8 ± 0.2	99.1	0.952	24	17.0 ± 0.2	100.2	1.000
40	24	17.1 ± 0.2	0.456	24	17.0 ± 0.2	99.5	0.995	24	16.8 ± 0.2	98.2	0.668	24	17.4 ± 0.2	102.0	0.586	24	17.1 ± 0.2	100.2	1.000
44	24	17.9 ± 0.4	0.944	24	17.6 ± 0.4	98.0	0.922	24	18.2 ± 0.4	101.3	0.981	24	17.7 ± 0.4	98.7	0.983	24	17.9 ± 0.4	99.9	1.000
48	24	19.1 ± 0.5	0.556	24	18.8 ± 0.5	98.5	0.980	24	19.4 ± 0.5	101.4	0.983	24	19.2 ± 0.5	100.4	1.000	24	19.4 ± 0.5	101.4	0.982
52	24	20.6 ± 0.4	0.611	24	20.7 ± 0.4	100.2	1.000	24	20.7 ± 0.4	100.3	1.000	24	20.4 ± 0.4	98.8	0.983	24	21.0 ± 0.4	101.7	0.937
56	24	21.2 ± 0.3	0.129	24	20.7 ± 0.3	97.3	0.374	24	20.5 ± 0.3	96.4	0.148	24	20.6 ± 0.3	97.1	0.320	24	20.4 ± 0.3	96.3	0.133
60	24	21.0 ± 0.3	0.665	24	21.1 ± 0.3	100.5	0.999	24	20.9 ± 0.3	99.4	0.996	24	20.9 ± 0.3	99.2	0.991	24	21.3 ± 0.3	101.1	0.969
64	24	21.2 ± 0.4	0.422	24	21.3 ± 0.4	100.2	1.000	24	21.1 ± 0.4	99.3	0.997	24	20.9 ± 0.4	98.7	0.976	23	21.7 ± 0.4	102.2	0.848
68	24	20.9 ± 0.5	0.942	24	21.4 ± 0.5	102.8	0.820	24	20.7 ± 0.5	99.2	0.998	24	21.2 ± 0.5	101.7	0.963	22	21.0 ± 0.5	100.8	0.997
72	24	21.3 ± 0.6	0.176	24	21.3 ± 0.6	100.2	1.000	24	20.3 ± 0.6	95.7	0.625	24	21.4 ± 0.6	100.6	1.000	22	20.1 ± 0.6	94.4	0.401
76	24	21.4 ± 0.6	0.587	24	21.0 ± 0.6	97.8	0.954	23	20.0 ± 0.6	93.4	0.284	24	20.3 ± 0.6	94.6	0.453	22	20.8 ± 0.6	96.9	0.863
80	24	21.7 ± 0.7	0.443	24	20.7 ± 0.7	95.6	0.715	23	19.4 ± 0.7	89.2	0.053	24	20.4 ± 0.7	94.1	0.475	21	21.8 ± 0.7	100.4	1.000
84	24	23.0 ± 0.8	0.682	24	21.6 ± 0.8	93.9	0.536	22	19.5 ± 0.8	85.0	0.009	22	21.5 ± 0.8	93.5	0.479	21	22.4 ± 0.8	97.7	0.974
88	24	23.2 ± 0.9	0.431	24	21.0 ± 0.9	90.5	0.254	21	20.1 ± 0.9	86.5	0.054	21	23.2 ± 0.9	99.7	1.000	17	22.7 ± 1.0	97.6	0.982
92	21	22.7 ± 1.2	0.136	21	20.6 ± 1.2	90.8	0.524	21	19.4 ± 1.2	85.5	0.153	20	22.9 ± 1.2	100.7	1.000	15	23.5 ± 1.3	103.6	0.972
96	21	23.8 ± 1.3	0.148	21	20.9 ± 1.3	87.7	0.312	18	20.6 ± 1.3	86.8	0.261	18	18.2 ± 1.3	76.5	0.009	11	20.5 ± 1.5	86.2	0.280
100	21	24.9 ± 1.2	0.001	17	23.0 ± 1.2	92.5	0.632	15	22.9 ± 1.3	92.0	0.602	11	17.0 ± 1.3	68.1	<0.001	6	19.4 ± 1.6	77.9	0.018
104	17	27.3 ± 1.7	<0.001	14	21.0 ± 1.7	77.0	0.031	13	23.8 ± 1.8	87.0	0.397	7	16.6 ± 2.0	61.0	<0.001	3	14.4 ± 2.7	52.8	<0.001

Week[a]	0.0 mM			0.0875 mM				0.175 mM				0.35 mM				0.70 mM			
	N[b]	Mean ± SE[c]	P-Value[d]	N	Mean ± SE	Pct[e]	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value
Mean for Weeks																			
4–104		20.0 ± 0.4			19.2 ± 0.4				19.0 ± 0.4				18.8 ± 0.4				19.0 ± 0.4		

[a]Week indicates the last week of a 4-week interval of daily food consumption, measured weekly by cage.
[b]N = Number of cages.
[c]Mean ± SE (g per day) = Estimated least squares mean and standard error.
[d]P-values in the 0.0 mM glycidamide column are the p-values for the trend test; p-values in the treatment columns are Dunnett's adjusted p-values for pairwise comparisons of the dose groups to the 0.0 mM glycidamide group.
[e]Pct = Ratio of the mean food consumption of the dose groups to the mean food consumption of the 0.0 mM glycidamide group, expressed as a percent.

Table G-2. Food Consumption of Female Rats in the Two-year Drinking Water Study of Glycidamide

Week[a]	0.0 mM			0.0875 mM				0.175 mM				0.35 mM				0.70 mM			
	N[b]	Mean ± SE[c]	P-Value[d]	N	Mean ± SE	Pct[e]	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value
4	24	12.0 ± 0.2	0.025	24	11.6 ± 0.2	96.6	0.176	24	11.6 ± 0.2	96.6	0.175	24	11.5 ± 0.2	96.0	0.080	24	11.4 ± 0.2	95.2	0.025
8	24	11.6 ± 0.1	0.022	24	11.3 ± 0.1	97.7	0.504	24	11.3 ± 0.1	97.4	0.407	24	11.0 ± 0.1	95.1	0.023	24	11.1 ± 0.1	95.8	0.071
12	24	11.2 ± 0.1	0.053	24	11.0 ± 0.1	98.6	0.867	24	11.0 ± 0.1	98.6	0.876	24	11.0 ± 0.1	98.1	0.699	24	10.8 ± 0.1	96.3	0.150
16	24	11.3 ± 0.1	0.003	24	11.1 ± 0.1	98.2	0.695	24	11.0 ± 0.1	98.1	0.667	24	10.9 ± 0.1	96.7	0.199	24	10.7 ± 0.1	94.9	0.014
20	24	11.4 ± 0.2	0.055	24	11.2 ± 0.2	98.1	0.712	24	11.1 ± 0.2	97.3	0.402	24	10.8 ± 0.2	95.0	0.028	24	11.0 ± 0.2	96.5	0.187
24	24	11.7 ± 0.2	0.011	24	11.3 ± 0.2	96.3	0.174	24	11.5 ± 0.2	97.9	0.669	24	11.2 ± 0.2	95.2	0.052	24	11.1 ± 0.2	94.6	0.021
28	24	11.8 ± 0.1	<0.001	24	11.6 ± 0.1	98.0	0.499	24	11.7 ± 0.1	98.6	0.804	24	11.5 ± 0.1	97.2	0.216	24	11.2 ± 0.1	94.3	0.001
32	24	12.1 ± 0.1	0.003	24	11.9 ± 0.1	98.6	0.832	24	11.7 ± 0.1	97.2	0.306	24	11.7 ± 0.1	97.0	0.248	24	11.4 ± 0.1	94.8	0.010
36	24	12.1 ± 0.1	0.026	24	12.0 ± 0.1	99.7	0.999	24	12.0 ± 0.1	99.6	0.997	24	11.8 ± 0.1	97.8	0.435	24	11.7 ± 0.1	97.0	0.197
40	24	12.3 ± 0.1	0.058	24	12.4 ± 0.1	100.3	0.998	24	12.1 ± 0.1	98.1	0.504	24	12.0 ± 0.1	96.9	0.112	24	12.1 ± 0.1	97.9	0.403
44	24	12.7 ± 0.2	0.259	24	12.8 ± 0.2	100.8	0.988	24	12.7 ± 0.2	99.8	1.000	24	12.6 ± 0.2	99.2	0.988	24	12.5 ± 0.2	98.2	0.836
48	24	14.0 ± 0.3	0.430	24	13.4 ± 0.3	96.1	0.562	24	13.8 ± 0.3	98.8	0.986	24	13.5 ± 0.3	96.5	0.641	24	13.5 ± 0.3	96.6	0.678
52	24	14.9 ± 0.3	0.827	24	14.6 ± 0.3	97.9	0.861	24	14.7 ± 0.3	98.9	0.984	24	14.8 ± 0.3	99.7	1.000	24	14.6 ± 0.3	98.5	0.949
56	24	15.7 ± 0.3	0.893	24	15.2 ± 0.3	96.6	0.539	24	15.6 ± 0.3	99.2	0.995	24	15.2 ± 0.3	97.1	0.656	24	15.5 ± 0.3	98.7	0.974
60	24	15.6 ± 0.4	0.935	24	15.6 ± 0.4	100.3	1.000	24	15.9 ± 0.4	101.9	0.949	24	15.4 ± 0.4	98.9	0.992	24	15.7 ± 0.4	101.0	0.996
64	24	16.0 ± 0.4	0.910	24	15.8 ± 0.4	98.9	0.991	24	16.0 ± 0.4	100.1	1.000	24	15.8 ± 0.4	98.6	0.979	24	16.0 ± 0.4	100.2	1.000
68	24	16.5 ± 0.4	0.528	24	15.6 ± 0.4	94.8	0.410	24	16.2 ± 0.4	98.7	0.989	24	16.5 ± 0.4	100.5	1.000	23	16.4 ± 0.4	99.6	1.000
72	24	16.2 ± 0.5	0.666	24	15.8 ± 0.5	97.2	0.925	24	16.2 ± 0.5	100.1	1.000	24	16.3 ± 0.5	100.7	1.000	23	15.7 ± 0.5	97.0	0.910
76	24	15.6 ± 0.4	0.585	24	15.7 ± 0.4	100.7	0.999	24	16.0 ± 0.4	102.8	0.858	24	15.9 ± 0.4	101.7	0.974	20	15.4 ± 0.4	98.7	0.990
80	24	16.0 ± 0.5	0.912	24	15.8 ± 0.5	98.8	0.997	23	16.9 ± 0.5	105.4	0.625	24	17.2 ± 0.5	107.3	0.350	19	15.8 ± 0.6	98.6	0.996
84	24	17.1 ± 0.6	0.079	24	16.3 ± 0.6	95.5	0.784	23	17.3 ± 0.6	101.0	0.999	24	17.3 ± 0.6	101.1	0.999	16	15.4 ± 0.7	89.9	0.165
88	24	17.0 ± 0.6	0.008	23	16.6 ± 0.7	97.4	0.971	23	17.3 ± 0.7	101.3	0.998	24	17.1 ± 0.6	100.2	1.000	13	14.5 ± 0.7	85.3	0.038
92	24	17.7 ± 0.7	0.366	23	16.5 ± 0.7	93.3	0.519	23	17.0 ± 0.7	95.6	0.821	23	16.8 ± 0.7	94.5	0.687	10	16.4 ± 0.8	92.6	0.533
96	24	17.7 ± 0.9	0.029	23	17.6 ± 0.9	99.3	1.000	22	17.5 ± 0.9	98.7	0.999	21	16.7 ± 0.9	94.0	0.828	8	20.9 ± 1.2	118.0	0.115
100	23	18.7 ± 0.8	0.318	22	17.8 ± 0.8	95.2	0.828	20	17.5 ± 0.8	93.9	0.684	16	17.0 ± 0.8	90.8	0.342	6	17.2 ± 1.1	91.9	0.630
104	21	18.4 ± 0.9	0.768	20	18.6 ± 0.9	100.6	1.000	20	17.4 ± 0.9	94.2	0.801	14	17.1 ± 0.9	92.8	0.684	2	19.1 ± 1.7	103.5	0.992

Week[a]	0.0 mM			0.0875 mM				0.175 mM				0.35 mM				0.70 mM			
	N[b]	Mean ± SE[c]	P-Value[d]	N	Mean ± SE	Pct[e]	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value
Mean for Weeks																			
4–104		14.5 ± 0.3			14.2 ± 0.3				14.3 ± 0.3				14.2 ± 0.3				14.1 ± 0.3		

[a]Week indicates the last week of a 4-week interval of daily food consumption, measured weekly by cage.
[b]N = Number of cages.
[c]Mean ± SE (g per day) = Estimated least squares mean and standard error.
[d]P-values in the 0.0 mM glycidamide column are the p-values for the trend test; p-values in the treatment columns are Dunnett's adjusted p-values for pairwise comparisons of the dose groups to the 0.0 mM glycidamide group.
[e]Pct = Ratio of the mean food consumption of the dose groups to the mean food consumption of the 0.0 mM glycidamide group, expressed as a percent.

Table G-3. Food Consumption of Male Mice in the Two-year Drinking Water Study of Glycidamide

Week[a]	0.0 mM			0.0875 mM				0.175 mM				0.35 mM				0.70 mM			
	N[b]	Mean ± SE[c]	P-Value[d]	N	Mean ± SE	Pct[e]	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value
4	12	2.9 ± 0.3	0.536	12	3.5 ± 0.3	119.1	0.387	12	3.4 ± 0.3	115.1	0.595	12	3.0 ± 0.3	103.4	0.997	12	3.0 ± 0.3	102.8	0.999
8	12	3.2 ± 0.1	0.428	12	3.2 ± 0.1	101.4	0.998	12	3.2 ± 0.1	101.0	1.000	12	3.2 ± 0.1	98.8	0.999	12	3.4 ± 0.1	105.5	0.796
12	12	3.5 ± 0.2	0.670	12	3.5 ± 0.2	99.0	1.000	12	3.5 ± 0.2	99.0	1.000	12	3.5 ± 0.2	98.8	0.999	12	3.4 ± 0.2	97.0	0.979
16	12	3.7 ± 0.1	0.637	12	3.7 ± 0.1	101.5	0.995	12	3.7 ± 0.1	99.4	1.000	12	3.8 ± 0.1	102.8	0.952	12	3.8 ± 0.1	102.1	0.983
20	12	4.1 ± 0.1	0.131	12	4.0 ± 0.1	99.1	0.989	12	4.0 ± 0.1	97.8	0.793	12	3.9 ± 0.1	96.6	0.467	12	4.2 ± 0.1	103.3	0.499
24	12	3.9 ± 0.1	0.665	12	3.9 ± 0.1	99.9	1.000	12	4.1 ± 0.1	104.3	0.405	12	3.9 ± 0.1	100.3	1.000	12	4.0 ± 0.1	101.9	0.920
28	12	3.9 ± 0.1	0.393	12	3.9 ± 0.1	100.7	0.997	12	3.9 ± 0.1	99.8	1.000	12	3.9 ± 0.1	101.5	0.942	12	3.9 ± 0.1	101.9	0.876
32	12	3.8 ± 0.1	0.271	12	3.8 ± 0.1	100.2	1.000	12	3.9 ± 0.1	102.2	0.840	12	3.9 ± 0.1	101.2	0.976	12	3.9 ± 0.1	103.0	0.649
36	12	3.8 ± 0.1	0.744	12	3.8 ± 0.1	100.4	1.000	12	3.8 ± 0.1	100.0	1.000	12	4.0 ± 0.1	105.4	0.245	12	3.8 ± 0.1	100.0	1.000
40	12	3.9 ± 0.1	0.322	12	3.8 ± 0.1	96.1	0.711	12	3.8 ± 0.1	95.9	0.679	12	3.7 ± 0.1	94.1	0.369	12	3.8 ± 0.1	95.3	0.571
44	12	3.8 ± 0.1	0.322	12	3.6 ± 0.1	96.1	0.484	12	3.8 ± 0.1	99.6	1.000	12	3.8 ± 0.1	99.5	0.999	12	3.6 ± 0.1	95.8	0.426
48	12	4.3 ± 0.3	0.550	12	3.7 ± 0.3	85.8	0.490	12	3.9 ± 0.3	89.8	0.752	12	3.8 ± 0.3	87.5	0.599	12	3.8 ± 0.3	89.0	0.700
52	12	3.7 ± 0.1	0.620	12	3.5 ± 0.1	94.4	0.375	12	3.7 ± 0.1	99.2	0.998	12	3.6 ± 0.1	97.1	0.855	12	3.7 ± 0.1	99.8	1.000
56	12	3.6 ± 0.1	0.939	12	3.6 ± 0.1	98.4	0.986	12	3.5 ± 0.1	96.5	0.799	12	3.7 ± 0.1	102.0	0.962	12	3.5 ± 0.1	98.0	0.965
60	12	3.4 ± 0.1	0.098	12	3.4 ± 0.1	99.3	0.999	12	3.6 ± 0.1	106.5	0.351	12	3.5 ± 0.1	102.0	0.972	12	3.6 ± 0.1	106.9	0.297
64	12	3.2 ± 0.1	0.173	12	3.4 ± 0.1	104.4	0.754	12	3.4 ± 0.1	105.6	0.577	12	3.5 ± 0.1	108.3	0.230	12	3.5 ± 0.1	106.9	0.381
68	12	3.4 ± 0.2	0.555	12	3.3 ± 0.2	96.4	0.944	12	3.6 ± 0.2	105.3	0.817	12	3.7 ± 0.2	106.0	0.743	12	3.5 ± 0.2	101.7	0.996
72	12	3.5 ± 0.2	0.124	12	3.6 ± 0.2	101.8	0.995	12	3.9 ± 0.2	109.1	0.396	12	4.0 ± 0.2	113.2	0.111	12	3.8 ± 0.2	108.5	0.455
76	12	3.7 ± 0.1	0.351	12	3.8 ± 0.1	102.8	0.916	12	3.8 ± 0.1	102.7	0.925	12	4.0 ± 0.1	107.4	0.265	12	3.9 ± 0.1	103.9	0.791
80	12	3.6 ± 0.2	0.332	12	3.8 ± 0.2	105.2	0.914	12	3.8 ± 0.2	105.4	0.905	12	4.2 ± 0.2	114.9	0.195	12	3.9 ± 0.2	107.2	0.783
84	12	3.5 ± 0.3	0.014	12	3.5 ± 0.3	100.7	1.000	12	4.0 ± 0.3	113.3	0.511	12	4.4 ± 0.3	126.0	0.041	12	4.2 ± 0.3	120.4	0.152
88	12	3.6 ± 0.3	0.001	12	3.6 ± 0.3	101.9	0.999	12	3.8 ± 0.3	107.9	0.885	12	4.5 ± 0.3	127.6	0.042	12	4.6 ± 0.3	128.6	0.033
92	12	4.0 ± 0.3	0.006	12	3.7 ± 0.3	92.3	0.846	12	4.3 ± 0.3	107.4	0.864	12	4.7 ± 0.3	117.7	0.212	12	4.8 ± 0.3	118.8	0.168
96	12	3.7 ± 0.4	<0.001	12	3.9 ± 0.4	106.6	0.977	12	4.7 ± 0.4	127.9	0.176	12	5.6 ± 0.4	151.7	0.002	12	5.4 ± 0.4	146.3	0.006
100	12	3.7 ± 0.6	0.004	12	4.1 ± 0.6	112.4	0.942	12	5.1 ± 0.6	138.8	0.209	12	6.1 ± 0.6	166.3	0.007	12	5.7 ± 0.6	155.1	0.035
104	12	3.7 ± 0.6	<0.001	12	4.1 ± 0.6	111.7	0.962	12	5.3 ± 0.6	143.5	0.176	12	6.0 ± 0.6	162.7	0.022	12	6.3 ± 0.6	172.4	0.006

Week[a]	0.0 mM			0.0875 mM				0.175 mM				0.35 mM				0.70 mM			
	N[b]	Mean ± SE[c]	P-Value[d]	N	Mean ± SE	Pct[e]	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value
Mean for Weeks																			
4–104		3.7 ± 0.1			3.7 ± 0.1				3.9 ± 0.1				4.1 ± 0.1				4.0 ± 0.1		

[a]Week indicates the last week of a 4-week interval of daily food consumption, measured weekly by cage.
[b]N = Number of cages.
[c]Mean ± SE (g per day) = Estimated least squares mean and standard error.
[d]P-values in the 0.0 mM glycidamide column are the p-values for the trend test; p-values in the treatment columns are Dunnett's adjusted p-values for pairwise comparisons of the dose groups to the 0.0 mM glycidamide group.
[e]Pct = Ratio of the mean food consumption of the dose groups to the mean food consumption of the 0.0 mM glycidamide group, expressed as a percent.

Table G-4. Food Consumption of Female Mice in the Two-year Drinking Water Study of Glycidamide

Week[a]	0.0 mM			0.0875 mM				0.175 mM				0.35 mM				0.70 mM			
	N[b]	Mean ± SE[c]	P-Value[d]	N	Mean ± SE	Pct[e]	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value
4	12	2.8 ± 0.1	0.533	12	2.8 ± 0.1	102.9	0.708	12	2.8 ± 0.1	102.4	0.809	12	2.7 ± 0.1	98.4	0.946	12	2.8 ± 0.1	100.4	1.000
8	12	3.0 ± 0.1	0.651	12	3.0 ± 0.1	99.8	1.000	12	3.0 ± 0.1	98.0	0.973	12	3.0 ± 0.1	99.0	0.998	12	3.0 ± 0.1	98.0	0.973
12	12	3.3 ± 0.2	0.683	12	3.5 ± 0.2	105.3	0.874	12	3.3 ± 0.2	100.6	1.000	12	3.3 ± 0.2	98.8	0.999	12	3.3 ± 0.2	99.9	1.000
16	12	3.7 ± 0.2	0.820	12	3.6 ± 0.2	99.5	1.000	12	3.5 ± 0.2	95.3	0.836	12	3.6 ± 0.2	99.0	0.999	12	3.7 ± 0.2	100.2	1.000
20	12	4.0 ± 0.1	0.715	12	4.0 ± 0.1	100.4	0.999	12	4.0 ± 0.1	100.1	1.000	12	3.9 ± 0.1	98.0	0.827	12	3.9 ± 0.1	99.8	1.000
24	12	3.8 ± 0.1	0.119	12	3.8 ± 0.1	100.0	1.000	12	3.9 ± 0.1	103.3	0.571	12	3.7 ± 0.1	98.2	0.909	12	3.7 ± 0.1	97.2	0.700
28	12	3.9 ± 0.1	0.102	12	3.8 ± 0.1	98.7	0.960	12	3.8 ± 0.1	98.7	0.964	12	3.8 ± 0.1	97.2	0.627	12	3.7 ± 0.1	96.1	0.340
32	12	3.8 ± 0.1	0.822	12	3.8 ± 0.1	98.9	0.979	12	3.9 ± 0.1	102.0	0.838	12	3.9 ± 0.1	102.2	0.774	12	3.8 ± 0.1	99.0	0.985
36	12	3.8 ± 0.1	0.089	12	3.8 ± 0.1	100.6	0.998	12	3.9 ± 0.1	101.4	0.960	12	3.8 ± 0.1	99.1	0.992	12	3.7 ± 0.1	96.6	0.526
40	12	3.8 ± 0.1	0.340	12	3.8 ± 0.1	99.9	1.000	12	3.8 ± 0.1	102.0	0.910	12	3.8 ± 0.1	101.4	0.969	12	3.7 ± 0.1	97.5	0.820
44	12	3.9 ± 0.1	0.353	12	3.7 ± 0.1	97.0	0.819	12	3.9 ± 0.1	101.5	0.981	12	3.9 ± 0.1	100.5	1.000	12	3.9 ± 0.1	102.0	0.950
48	12	3.8 ± 0.1	0.467	12	3.9 ± 0.1	102.3	0.922	12	3.8 ± 0.1	100.1	1.000	12	3.8 ± 0.1	100.9	0.998	12	3.9 ± 0.1	103.2	0.803
52	12	3.8 ± 0.1	0.413	12	3.7 ± 0.1	96.7	0.863	12	3.7 ± 0.1	97.2	0.917	12	3.9 ± 0.1	102.0	0.973	12	3.8 ± 0.1	100.9	0.999
56	12	3.6 ± 0.2	0.993	12	4.1 ± 0.2	114.6	0.278	12	3.7 ± 0.2	102.7	0.994	12	3.8 ± 0.2	105.5	0.925	12	3.8 ± 0.2	105.2	0.937
60	12	3.6 ± 0.1	0.036	12	3.5 ± 0.1	97.5	0.928	12	3.5 ± 0.1	99.3	0.999	12	3.5 ± 0.1	99.1	0.998	12	3.8 ± 0.1	106.6	0.287
64	12	3.5 ± 0.1	0.011	12	3.3 ± 0.1	96.6	0.809	12	3.5 ± 0.1	100.5	1.000	12	3.5 ± 0.1	102.5	0.919	12	3.7 ± 0.1	107.3	0.200
68	12	3.4 ± 0.2	0.002	12	3.4 ± 0.2	99.8	1.000	12	3.6 ± 0.2	105.7	0.825	12	3.6 ± 0.2	104.0	0.944	12	4.1 ± 0.2	119.3	0.019
72	12	3.7 ± 0.3	0.006	12	3.6 ± 0.3	96.7	0.995	12	3.7 ± 0.3	100.2	1.000	12	3.9 ± 0.3	103.7	0.992	12	4.7 ± 0.3	125.1	0.078
76	12	3.9 ± 0.4	<0.001	12	3.8 ± 0.4	95.9	0.993	12	4.1 ± 0.4	105.0	0.984	12	4.1 ± 0.4	103.7	0.995	12	5.6 ± 0.4	141.3	0.004
80	12	3.8 ± 0.3	<0.001	12	4.0 ± 0.3	104.2	0.982	12	4.0 ± 0.3	104.1	0.983	12	4.2 ± 0.3	109.8	0.731	12	5.3 ± 0.3	137.5	0.001
84	12	3.9 ± 0.3	<0.001	12	4.0 ± 0.3	101.2	1.000	12	4.0 ± 0.3	101.2	1.000	12	4.1 ± 0.3	104.8	0.980	12	6.4 ± 0.3	163.6	<0.001
88	12	3.8 ± 0.3	<0.001	12	3.9 ± 0.3	103.4	0.996	12	4.2 ± 0.3	111.8	0.737	12	4.5 ± 0.3	120.0	0.299	12	6.9 ± 0.3	181.2	<0.001
92	12	4.2 ± 0.5	<0.001	12	4.0 ± 0.5	95.6	0.995	12	4.4 ± 0.5	104.7	0.994	12	4.5 ± 0.5	105.6	0.989	11	7.6 ± 0.5	178.6	<0.001
96	12	4.0 ± 0.4	<0.001	12	4.2 ± 0.4	105.3	0.988	12	4.7 ± 0.4	116.1	0.620	12	4.8 ± 0.4	118.8	0.487	11	8.5 ± 0.4	212.0	<0.001
100	12	4.2 ± 0.4	<0.001	12	4.3 ± 0.4	103.0	0.999	12	4.8 ± 0.4	112.7	0.777	12	5.2 ± 0.4	124.2	0.251	9	9.5 ± 0.4	224.0	<0.001
104	12	4.4 ± 0.4	<0.001	12	4.3 ± 0.4	97.3	0.998	12	4.8 ± 0.4	108.5	0.897	12	5.6 ± 0.4	128.4	0.073	7	10.3 ± 0.4	235.4	<0.001

Week[a]	0.0 mM			0.0875 mM				0.175 mM				0.35 mM				0.70 mM			
	N[b]	Mean ± SE[c]	P-Value[d]	N	Mean ± SE	Pct[e]	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value	N	Mean ± SE	Pct	P-Value
Mean for Weeks																			
4–104		3.7 ± 0.1			3.8 ± 0.1				3.9 ± 0.1				3.9 ± 0.1				4.9 ± 0.1		

[a]Week indicates the last week of a 4-week interval of daily food consumption, measured weekly by cage.
[b]N = Number of cages.
[c]Mean ± SE (g per day) = Estimated least squares mean and standard error.
[d]P-values in the 0.0 mM glycidamide column are the p-values for the trend test; p-values in the treatment columns are Dunnett's adjusted p-values for pairwise comparisons of the dose groups to the 0.0 mM glycidamide group.
[e]Pct = Ratio of the mean food consumption of the dose groups to the mean food consumption of the 0.0 mM glycidamide group, expressed as a percent.

Appendix H. Ingredients, Nutrient Composition, and Contaminant Levels in NIH-31 Rat and Mouse Ration

Tables

Table H-1. Ingredients of NIH-31 IR Rat and Mouse Ration

Ingredients[a]	Percent by Weight
Ground whole hard wheat	35.5
Ground #2 yellow shelled corn	21.0
Ground whole oats	10.0
Wheat middlings	10.0
Fish meal (60% protein)	9.0
Soybean meal (48.5% protein)	5.0
Alfalfa meal (17% protein)	2.0
Corn gluten meal (60%)	2.0
Dicalcium phosphate[b]	1.5
Soy oil	1.5
Brewers dried yeast	1.0
Ground limestone[b]	0.5
Premixes	0.5
Salt	0.5

[a]Ingredients ground to pass through a U.S. Standard Screen No. 16 before mixing.
[b]Specific ingredient requirement for cadmium content not to exceed 1 mg/kg.

Table H-2. Vitamins and Minerals in NIH-31 IR Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	22,000,000 IU	Vitamin A palmitate or acetate
D_3	3,800,000 IU	D-activated animal sterol
K_3	20 g	Menadione activity
Choline	700 g	Choline chloride
dl-α-Tocopheryl acetate	15 g	–
Folic acid	1 g	–
Niacin	20 g	–
d-Pantothenic acid	25 g	*d*-Calcium pantothenate
Riboflavin	5 g	–
Thiamine	65 g	Thiamine mononitrate
B_{12}	14 g	–
Pyridoxine	2 g	Pyridoxine hydrochloride
Biotin	0.12 g	*d*-Biotin
Minerals		
Magnesium	400 g	Magnesium oxide
Manganese	100 g	Manganese oxide

	Amount	Source
Iron	60 g	Iron sulfate
Zinc	10 g	Zinc oxide
Copper	4 g	Copper sulfate
Iodine	1.5 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a]Per ton (2000 lb) of finished product.

Table H-3. Nutrient Composition of NIH-31 IR Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Number of Lots
Crude protein (% by weight)	18.3 ± 0.5	10
Crude fat (% by weight)	6.24 ± 0.60	10
Volatiles (% by weight)	8.50 ± 0.49	10
Vitamins		
A (µg/g)	3.09 ± 0.57	10
E (µg/g)	38.5 ± 11.9	10
B1 (µg/g)	26.0 ± 0.03	10
Minerals		
Selenium (µg/g)	0.39 ± 0.08	10

Table H-4. Contaminant Levels in NIH-31 IR Rat and Mouse Ration

	Mean ± Standard Deviation	Number of Samples (Number Positive)
Contaminants		
Acrylamide (ppb)	28.1 ± 24.7	10 (9)
Arsenic (µg/g)	0.18 ± 0.02	10 (10)
Cadmium (µg/g)	0.19 ± 0.07	10 (10)
Lead (µg/g)	0.42 ± 0.09	10 (10)
Aflatoxin B1 (ppb)	<mdl	10 (0)
Aflatoxin B2 (ppb)	<mdl	10 (0)
Aflatoxin G1 (ppb)	<mdl	10 (0)
Aflatoxin G2 (ppb)	<mdl	10 (0)
Total Fumonisin (ppb)	343 ± 213	10 (10)

	Mean ± Standard Deviation	Number of Samples (Number Positive)
Pesticides (ppb)		
Heptachlor	<mdl	1 (0)
Total DDT	<mdl	1 (0)
Dieldrin	<mdl	1 (0)
PCB	<mdl	1 (0)
Malathion	<mdl	1 (0)
Lindane	<mdl	1 (0)

Appendix I. Sentinel Animal Program

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Tables

I.1. Methods

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

Blood from each sentinel animal was collected, allowed to clot, and the serum was separated. The serum was analyzed by Multiplex Fluorescent Immunoassay (MFI) for the presence of specific antibodies by the Research Animal Diagnostic Laboratory, University of Missouri, Columbia, Missouri. The laboratory serology method and viral/mycoplasma agent for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Table I-1. Laboratory Methods and Agents Tested for in the Sentinel Animal Program[a]

Method and Test	Time of Analysis
Mice	
MFI	
Mouse Hepatitis Virus (MHV)	26, 53, 79, 104, & 117 weeks
Sendai	26, 53, 79, 104, & 117 weeks
Pneumonia Virus of Mice (PVM)	26, 53, 79, 104, & 117 weeks
Reovirus Type 3 (REO3)	26, 53, 79, 104, & 117 weeks
Theiler's Murine Encephalomyelitis Virus (TMEV)	26, 53, 79, 104, & 117 weeks
Ectromelia	26, 53, 79, 104, & 117 weeks
Polyoma	26, 53, 79, 104, & 117 weeks
Mycoplasma pulmonis	26, 53, 79, 104, & 117 weeks
Minute Virus of Mice (MMV)	26, 53, 79, 104, & 117 weeks
Mouse Parvovirus (MPV)	26, 53, 79, 104, & 117 weeks
Parvo NS-1	26, 53, 79, 104, & 117 weeks
Epizootic Diarrhea of Infant Mice Virus (EDIM)	26, 53, 79, 104, & 117 weeks
Lymphocytic Choriomeningitis Virus (LCM)	26, 53, 79, 104, & 117 weeks
Rats	
MFI	
Rat Coronavirus/Sialodacryoadenitis (RCV/SDAV)	26, 53, 79, & 104 weeks
Sendai	26, 53, 79, & 104 weeks
Pneumonia Virus of Mice (PVM)	26, 53, 79, & 104 weeks
TMEV GDVII	26, 53, 79, & 104 weeks

Method and Test		Time of Analysis
Mycoplasma pulmonis		26, 53, 79, & 104 weeks
Parvo NS-1		26, 53, 79, & 104 weeks
Rats and Mice		
Additional Screening		
Bordetella bronchiseptica	*Listeria monocytogenes*	Ectoparasites
Citrobacter freundii	*Pasteurella pneumontropica*	Endoparasites
Corynebacterium kutscheri	*Pasteurella multocida*	
Erysipelothrix rhusiopathiae	*Pseudomonas aeruginosa*	
Helicobacter bilis	*Salmonella sp.*	
Helicobacter hepaticus	*Streptococcus pneumoniae*	

[a]One on-test mouse and five on-test rats were screened in addition to sentinel animals.

I.2. Results

All serology test results were negative.

Helicobacter hepaticus was detected via polymerase chain reaction (PCR) in three of the sentinel mice. *Pseudomonas aeruginosa* was detected in two of the sentinel rats.

Appendix J. Genetic Toxicology

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J.1. Methods

J.1.1. Bacterial Mutagenicity Assay

Testing was performed as reported by Zeiger et al.[75]. Glycidamide was sent to the testing laboratory and coded prior to testing. It was incubated with the *Salmonella typhimurium* tester strains (TA98, TA100) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rats) for 20 minutes at 37°C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following a 2-day incubation at 37°C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of glycidamide. In the absence of toxicity, the high dose was limited to 10,000 μg/plate. Trials that gave a positive response were repeated.

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

J.2. Results

Glycidamide (concentration range, 100–10,000 μg/plate) was strongly mutagenic in *Salmonella typhimurium* strain TA100, with and without exogenous metabolic activation (S9 mix); addition of S9 slightly enhanced the mutagenic response compared to the trials conducted without S9, but the mutagenic response was extremely strong under both activation conditions (Table J-1). In contrast, no significant mutagenic activity was seen in strain TA98, with or without S9.

Table J-1. Mutagenicity of Glycidamide in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
Study performed at Bioreliance Corporation					
TA100					
	0	148 ± 21.0	154 ± 13.0	149 ± 6.0	176 ± 3.0
	100	192 ± 17.0	232 ± 8.0	184 ± 22.0	238 ± 5.0
	333	307 ± 6.0	512 ± 40.0	373 ± 7.0	400 ± 41.0
	1000	613 ± 26.0	589 ± 129.0	621 ± 92.0	1,024 ± 15.0
	3333	1,022 ± 15.0	1,267 ± 18.0	1,055 ± 22.0	1,193 ± 80.0
	10000	1,210 ± 42.0	1,591 ± 24.0	1,534 ± 55.0	1,730 ± 127.0
Trial summary		Positive	Positive	Positive	Positive
Positive control[b]		538 ± 9.0	532 ± 38.0	1,027 ± 34.0	693 ± 13.0
TA98					
	0	16 ± 2.0		30 ± 2.0	
	100	22 ± 1.0		36 ± 3.0	
	333	18 ± 2.0		27 ± 0.0	
	1000	21 ± 3.0		33 ± 1.0	
	3333	26 ± 1.0		37 ± 2.0	
	10000	27 ± 1.0		34 ± 7.0	
Trial summary		Negative		Negative	
Positive control		76 ± 25.0		581 ± 5.0	

[a]Data are presented as revertants/plate (mean ± standard error) from three plates. 0 µg/plate was the solvent control.
[b]The positive controls in the absence of metabolic activation were sodium azide (TA100) and 4-nitro-o-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Appendix K. Benchmark Dose Modeling

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K.1. Methods

K.1.1. Benchmark Dose Estimations

Benchmark doses (BMD) and the lower (BMDL) 95% confidence limits were calculated using Environmental Protection Agency Benchmark Dose Software (version 2.1.1; http://www.epa.gov/ncea/bmds). The calculations were conducted using gamma, logistic, log-logistic, log-probit, multistage, probit, and Weibull models to fit the neoplastic incidences and the mean doses of glycidamide for the entire 2-year study. The BMD was defined as the dose that caused a 10% excess risk of the specified adverse effect over that observed in the appropriate control group. Models were rejected if 1) $p < 0.05$ for the fitted model versus the full model; 2) $p < 0.05$ for the goodness of fit; or 3) The scaled residual for one or more of the doses in goodness of fit determination was $>|2.00|$[76]. In some instances the BMD calculations failed. If a model was rejected, the results are not reported.

K.2. Results

Benchmark dose modeling was conducted to estimate the doses of glycidamide that would give a 10% excess risk for specific neoplasms (BMD). In F344/N Nctr rats, the most sensitive site for tumor induction was the mammary gland in females (BMD of 2.39 µmol glycidamide per kg body weight per day for fibroadenoma) and the thyroid gland in males (BMD of 18.89–22.62 µmol glycidamide per kg body weight per day for combined follicular cell adenoma or carcinoma) (Table K-1). In B6C3F1/Nctr mice, the most sensitive site for tumor induction by glycidamide was the Harderian gland, with a BMD for Harderian gland adenoma of 5.24–5.91 µmol glycidamide per kg body weight per day in males and 4.55 µmol glycidamide per kg body weight per day in females (Table K-2).

A comparison of the glycidamide data with those previously reported for acrylamide[74] indicates that both chemicals have similar potencies in the target tissues (Table K-3 and Table K-4). For example, in female F344/N Nctr rats, the BMD for mammary gland fibroadenoma was 2.39 µMol per kg body weight per day for glycidamide and 7.74 µMol per kg body weight per day for acrylamide, and in male F344/N Nctr rats, the BMD for follicular cell adenoma or carcinoma of the thyroid gland was 19.33–22.62 µMol per kg body weight per day for glycidamide and 19.41–28.19 µMol per kg body weight per day for acrylamide (Table K-3). The BMD for Harderian gland adenoma in male B6C3F1/Nctr mice was 5.51–5.91 µMol per kg body weight per day for glycidamide as compared to 5.14–5.39 µMol per kg body weight per day for acrylamide (Table K-4). Likewise, in female B6C3F1/Nctr mice the BMD for Harderian gland adenoma was 4.55 µMol per kg body weight per day for glycidamide as compared to 6.55 µMol per kg body weight per day for acrylamide.

Table K-1. Benchmark Dose Modeling of Neoplastic Incidences in F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide[a]

	AIC[b]	Fitted Model[c]	GOF[d]	BMD[e]	BMDL[f]
Thyroid Gland					
Carcinoma					
Male					
Gamma	88.1	0.232	0.294	40.32	19.71
Logistic	88.5	0.202	0.300	42.24	29.43
Log-Logistic	88.1	0.236	0.294	39.76	18.96
Log-Probit	86.3	0.475	0.560	70.34	17.40
Multistage	88.1	0.232	0.294	40.32	19.71
Probit	88.4	0.203	0.300	42.65	28.45
Weibull	88.1	0.232	0.294	40.32	19.71
Adenoma or Carcinoma					
Male					
Gamma	164.6	0.338	0.350	19.33	10.56
Logistic	162.3	0.611	0.603	22.62	17.85
Log-Logistic	164.7	0.332	0.336	20.77	9.75
Log-Probit	164.9	0.291	0.307	18.89	6.42
Multistage	164.4	0.378	0.381	20.85	10.75
Probit	162.3	0.602	0.599	21.65	16.73
Weibull	164.6	0.345	0.350	20.50	10.60
Female					
Gamma	132.2	0.256	0.294	27.00	16.20
Logistic	134.0	0.118	0.251	42.74	31.72
Log-Logistic	131.9	0.293	0.300	24.05	14.61
Multistage	132.2	0.256	0.294	27.00	16.20
Probit	133.8	0.127	0.257	41.31	29.90
Weibull	132.2	0.256	0.294	27.00	16.20
Heart					
Malignant Schwannoma					
Male					
Gamma	149.2	0.829	0.821	25.06	14.24
Logistic	149.9	0.672	0.644	31.09	22.19
Log-Logistic	149.2	0.841	0.835	24.40	13.30
Log-Probit	151.1	0.689	0.687	23.23	8.60
Multistage	149.2	0.829	0.821	25.06	14.24

	AIC[b]	Fitted Model[c]	GOF[d]	BMD[e]	BMDL[f]
Probit	149.8	0.699	0.674	30.17	20.97
Weibull	149.2	0.829	0.821	25.06	14.24
Epididymis or Testes					
Malignant Mesothelioma					
Male					
Gamma	148.2	0.801	0.794	11.71	7.80
Log-Logistic	148.1	0.840	0.835	11.56	7.53
Log-Probit	147.7	0.916	0.913	11.23	7.61
Multistage	148.7	0.691	0.691	11.59	7.58
Probit	153.6	0.094	0.128	17.94	15.01
Weibull	148.3	0.778	0.772	11.69	7.75
Mammary Gland					
Fibroadenoma					
Female					
Log-Logistic	306.5	0.140	0.141	2.39	1.39
Oral Mucosa or Tongue					
Squamous Cell Papilloma or Carcinoma					
Female					
Gamma	105.9	0.801	0.814	50.94	29.59
Logistic	104.0	0.916	0.917	48.94	36.92
Log-Logistic	105.9	0.803	0.815	51.03	29.29
Log-Probit	105.9	0.799	0.813	50.82	28.90
Multistage	106.1	0.753	0.758	49.97	29.20
Probit	104.1	0.902	0.906	48.73	35.39
Weibull	105.9	0.803	0.815	51.09	29.60

[a]Benchmark doses (BMD) and the lower (BMDL) 95% confidence limits, in units of mg per kg body weight per day, were calculated using Environmental Protection Agency Benchmark Dose Software (version 2.1.1; http://www.epa.gov/ncea/bmds). The calculations were conducted using gamma, logistic, log-logistic, log-probit, multistage, probit, and Weibull models to fit the neoplastic incidences and the mean doses of glycidamide from the entire 2-year study. The BMD was defined as the dose that caused a 10% excess risk in the specified adverse effect over that observed in the appropriate control group. Models were rejected if (1) $p < 0.05$ for the fitted model versus the full model; (2) $p < 0.05$ for the goodness of fit; or (3) the scaled residual for one or more of the doses in goodness of fit determination was $>|2.00|$[76]. In some instances the BMD calculations failed. If a model was rejected, the results are not reported.
[b]AIC, Akaike information criterion.
[c]p-value of fitted model compared to the full model.
[d]GOF, Goodness of fit p-value.
[e]BMD, benchmark dose (µmol glycidamide per kg body weight per day).
[f]BMDL, lower 95% confidence limit of benchmark dose (µmol glycidamide per kg body weight per day).

Table K-2. Benchmark Dose Modeling of Neoplastic Incidences in B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide[a]

	AIC[b]	Fitted Model[c]	GOF[d]	BMD[e]	BMDL[f]
Harderian Gland					
Adenoma					
Male					
Gamma	242.4	0.784	0.778	5.24	4.32
Log-Logistic	245.1	0.418	0.425	5.51	2.49
Log-Probit	244.9	0.459	0.464	5.91	2.49
Multistage	242.4	0.784	0.778	5.24	4.32
Weibull	242.4	0.784	0.778	5.24	4.32
Female					
Log-Logistic	253.5	0.099	0.100	4.55	3.39
Lung					
Alveolar/Bronchiolar Adenoma					
Male					
Log-Logistic	200.9	0.540	0.454	17.16	12.83
Log-Probit	200.8	0.769	0.804	9.41	0.71[g]
Female					
Gamma	167.9	0.554	0.570	99.36	51.88
Logistic	166.0	0.734	0.742	112.72	77.57
Log-Logistic	167.9	0.555	0.570	99.08	48.23
Log-Probit	167.9	0.552	0.563	100.00	25.29
Multistage	167.9	0.554	0.571	98.98	51.88
Probit	166.0	0.742	0.750	110.13	73.58
Weibull	167.9	0.554	0.570	99.33	51.88
Stomach (Forestomach)					
Squamous Cell Papilloma					
Male					
Gamma	106.0	0.470	0.536	52.55	36.13
Logistic	108.2	0.287	0.390	76.50	63.10
Log-Logistic	108.1	0.298	0.355	51.96	34.39
Log-Probit	108.5	0.256	0.310	49.72	28.18
Multistage	107.9	0.334	0.373	55.10	36.51
Probit	108.3	0.280	0.375	73.15	59.15
Weibull	106.0	0.470	0.536	52.55	36.13

	AIC[b]	Fitted Model[c]	GOF[d]	BMD[e]	BMDL[f]
Mammary Gland					
Adenoacanthoma					
Female					
Gamma	56.0	0.973	0.987	117.96	93.37
Logistic	56.8	0.803	0.858	129.97	112.32
Log-Logistic	56.1	0.963	0.982	119.46	93.94
Log-Probit	56.1	0.963	0.982	115.08	89.64
Multistage	55.1	0.860	0.938	116.96	90.94
Probit	56.5	0.871	0.912	125.67	106.43
Weibull	56.1	0.960	0.980	120.56	95.40
Adenocarcinoma					
Female					
Gamma	140.8	0.226	0.212	59.73	38.68
Logistic	141.7	0.119	0.087	89.16	72.35
Log-Logistic	140.7	0.242	0.230	58.43	36.20
Log-Probit	140.2	0.308	0.294	56.99	34.86
Multistage	141.0	0.200	0.201	56.03	38.07
Probit	141.1	0.155	0.118	83.45	67.03
Weibull	140.8	0.220	0.208	59.37	38.54
Adenoacanthoma or Adenocarcinoma					
Female					
Gamma	149.6	0.533	0.530	53.46	35.44
Logistic	150.1	0.283	0.255	71.73	59.97
Log-Logistic	149.5	0.554	0.554	52.72	35.13
Log-Probit	149.1	0.691	0.692	51.98	35.27
Multistage	150.2	0.392	0.386	55.63	33.92
Probit	149.3	0.391	0.364	66.26	55.26
Weibull	149.8	0.482	0.481	53.41	34.90

[a]Benchmark doses (BMD) and the lower (BMDL) 95% confidence limits, in units of mg per kg body weight per day, were calculated using Environmental Protection Agency Benchmark Dose Software (version 2.1.1; http://www.epa.gov/ncea/bmds). The calculations were conducted using gamma, logistic, log-logistic, log-probit, multistage, probit, and Weibull models to fit the neoplastic incidences and the mean doses of glycidamide from the entire 2-year study. The BMD was defined as the dose that caused a 10% excess risk in the specified adverse effect over that observed in the appropriate control group. Models were rejected if (1) $p < 0.05$ for the fitted model versus the full model; (2) $p < 0.05$ for the goodness of fit; or (3) The scaled residual for one or more of the doses in goodness of fit determination was $>|2.00|$[76]. In some instances the BMD calculations failed. If a model was rejected, the results are not reported.
[b]AIC, Akaike information criterion.
[c]p-value of fitted model compared to the full model.
[d]GOF, Goodness of fit p-value.
[e]BMD, benchmark dose (μmol glycidamide per kg body weight per day).
[f]BMDL, lower 95% confidence limit of benchmark dose (μmol glycidamide per kg body weight per day).

[g]Due to the disparity between the BMDL obtained from the log-probit model and the BMDL obtained from the other model, the log-probit BMDL value was not considered further[76].

Table K-3. Comparison of BMD for Selected Neoplasms in F344/N Nctr Rats Administered Glycidamide or Acrylamide in Two-year Drinking Water Studies

	Glycidamide (BMD, µmol/kg body weight/day)[a]	Acrylamide (BMD, µmol/kg body weight/day)[b]
Thyroid Gland		
Follicular Cell Carcinoma		
Male		
Gamma	40.32	28.57
Logistic	42.24	35.95
Log-Logistic	39.76	27.87
Log-Probit	70.34	26.72
Multistage	40.32	28.57
Probit	42.65	35.00
Weibull	40.32	28.57
Follicular Cell Adenoma or Carcinoma		
Male		
Gamma	19.33	20.37
Logistic	22.62	28.19
Log-Logistic	20.77	19.47
Multistage	20.85	20.37
Probit	21.65	27.14
Weibull	20.50	20.37
Female		
Gamma	27.00	54.15
Logistic	42.74	60.24
Log-Logistic	24.05	54.80
Multistage	27.00	54.15
Probit	41.31	59.36
Weibull	27.00	54.15
Heart		
Malignant Schwannoma		
Male		
Gamma	25.06	34.13
Logistic	31.09	37.68
Log-Logistic	24.40	33.91

	Glycidamide (BMD, µmol/kg body weight/day)[a]	Acrylamide (BMD, µmol/kg body weight/day)[b]
Log-Probit	23.23	34.76
Multistage	25.06	34.13
Probit	30.17	37.23
Weibull	25.06	34.13
Epididymis or Testes		
Malignant Mesothelioma		
Male		
Gamma	11.71	29.90
Multistage	11.59	30.66
Probit	17.94	30.36
Weibull	11.69	30.06
Mammary Gland		
Fibroadenoma		
Female		
Log-Logistic	2.39	7.74
Oral Mucosa or Tongue		
Squamous Cell Papilloma or Carcinoma		
Female		
Gamma	50.94	49.49
Logistic	48.94	58.01
Log-Logistic	51.03	48.42
Log-Probit	50.82	61.93
Multistage	49.97	49.49
Probit	48.73	57.54
Weibull	51.09	49.49

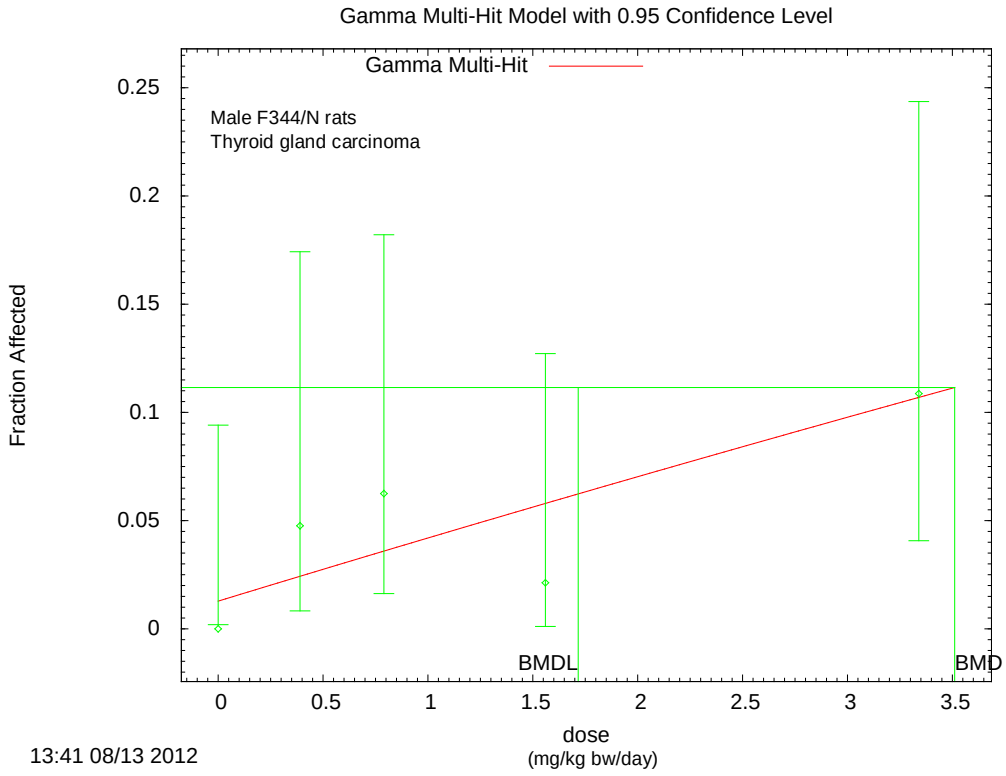
[a]The BMD for glycidamide in µMol/kg body weight/day are from the data presented in Table K-1.
[b]The BMD for acrylamide in µMol/kg body weight/day are calculated from the data presented in Beland et al.[74].

Table K-4. Comparison of BMD for Selected Neoplasms in B6C3F1/Nctr Mice Administered Glycidamide or Acrylamide in Two-year Drinking Water Studies

	Glycidamide (BMD, µmol/kg body weight/day)[a]	Acrylamide (BMD, µmol/kg body weight/day)[b]
Harderian Gland		
Adenoma		
Male		
Log-Logistic	5.51	5.14
Log-Probit	5.91	5.39
Female		
Log-Logistic	4.55	6.65
Lung		
Alveolar/Bronchiolar Adenoma		
Male		
Log-Logistic	17.16	29.59
Female		
Gamma	99.36	27.69
Logistic	112.72	56.06
Log-Logistic	99.08	27.15
Log-Probit	100.00	26.89
Multistage	98.98	27.69
Probit	110.13	51.97
Weibull	99.33	27.69
Stomach (Forestomach)		
Squamous Cell Papilloma		
Male		
Gamma	52.55	63.98
Logistic	76.50	105.74
Log-Logistic	51.96	62.30
Log-Probit	49.72	60.51
Multistage	55.10	63.98
Probit	73.15	101.39
Weibull	52.55	63.98
Mammary Gland		
Adenoacanthoma		
Female		
Gamma	117.96	140.56

	Glycidamide (BMD, µmol/kg body weight/day)[a]	Acrylamide (BMD, µmol/kg body weight/day)[b]
Logistic	129.97	146.10
Log-Logistic	119.46	142.80
Log-Probit	115.08	172.55
Multistage	116.96	140.56
Probit	125.67	145.74
Weibull	120.56	140.56
Adenoacanthoma or Adenocarcinoma		
Female		
Gamma	53.46	31.22
Log-Logistic	52.72	28.31
Log-Probit	51.98	24.23
Multistage	55.63	31.22
Weibull	53.41	31.22

[a]The BMD for glycidamide in µMol/kg body weight/day are from the data presented in Table K-2.
[b]The BMD for acrylamide in µMol/kg body weight/day are calculated from the data presented in Beland et al.[74].



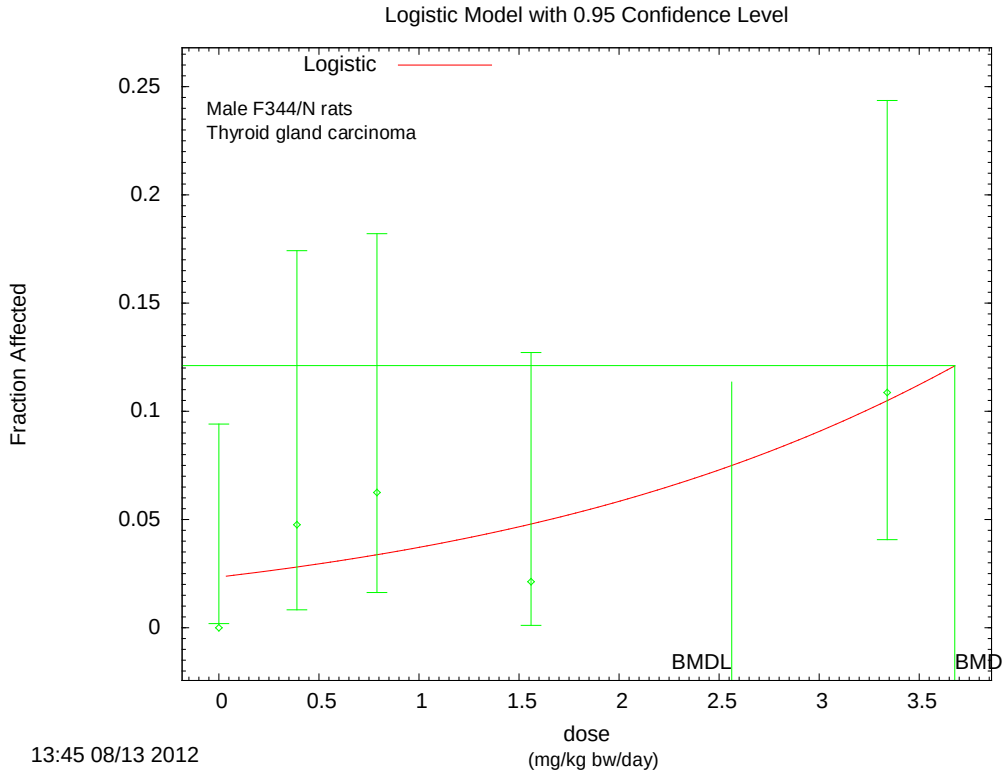


Figure K-1. Benchmark Dose Modeling of Thyroid Gland Carcinoma in Male F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Gamma Multi-hit Model; Bottom: Logistic Model)

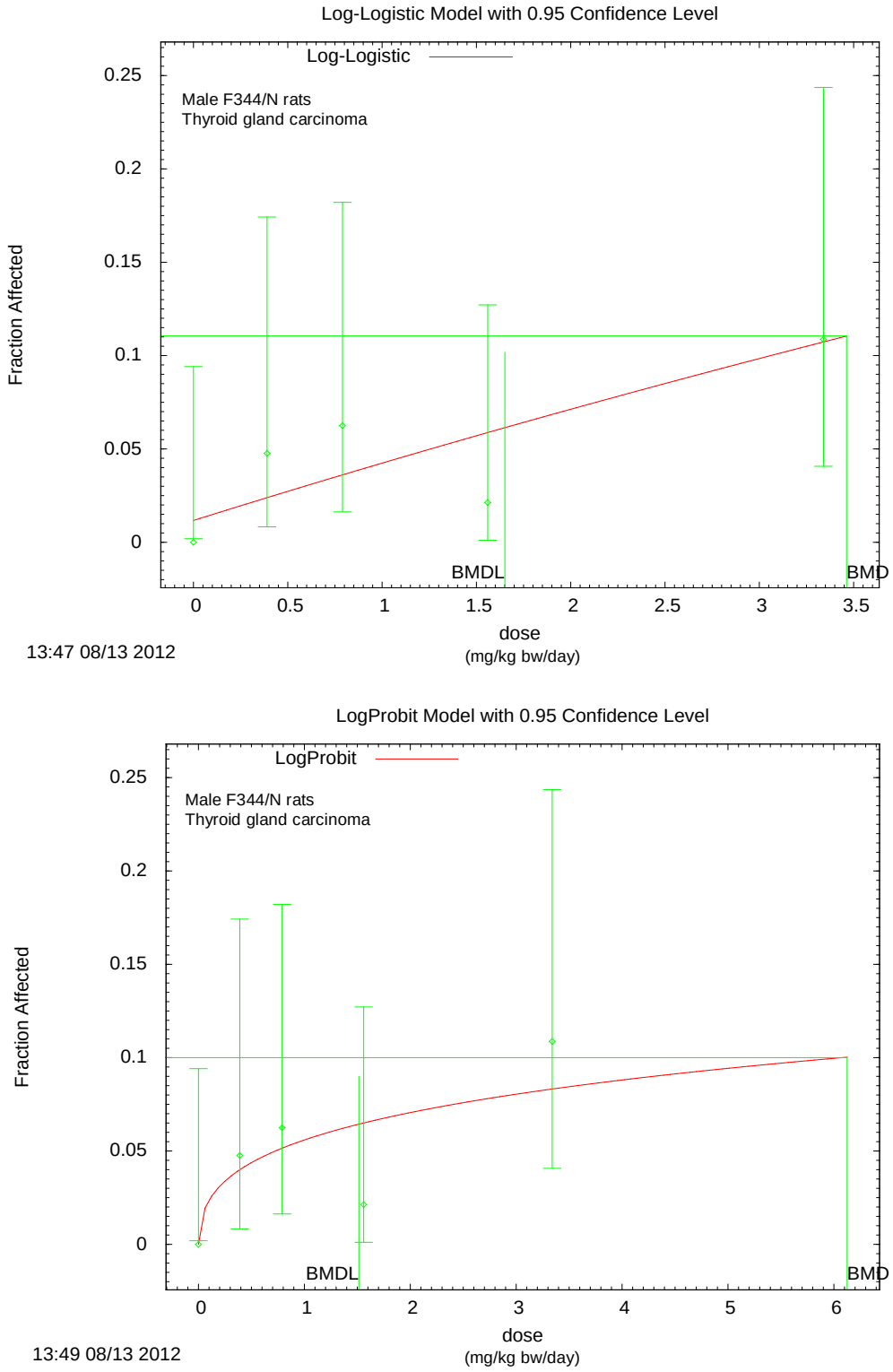


Figure K-2. Benchmark Dose Modeling of Thyroid Gland Carcinoma in Male F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Log-Logistic Model; Bottom: LogProbit Model)

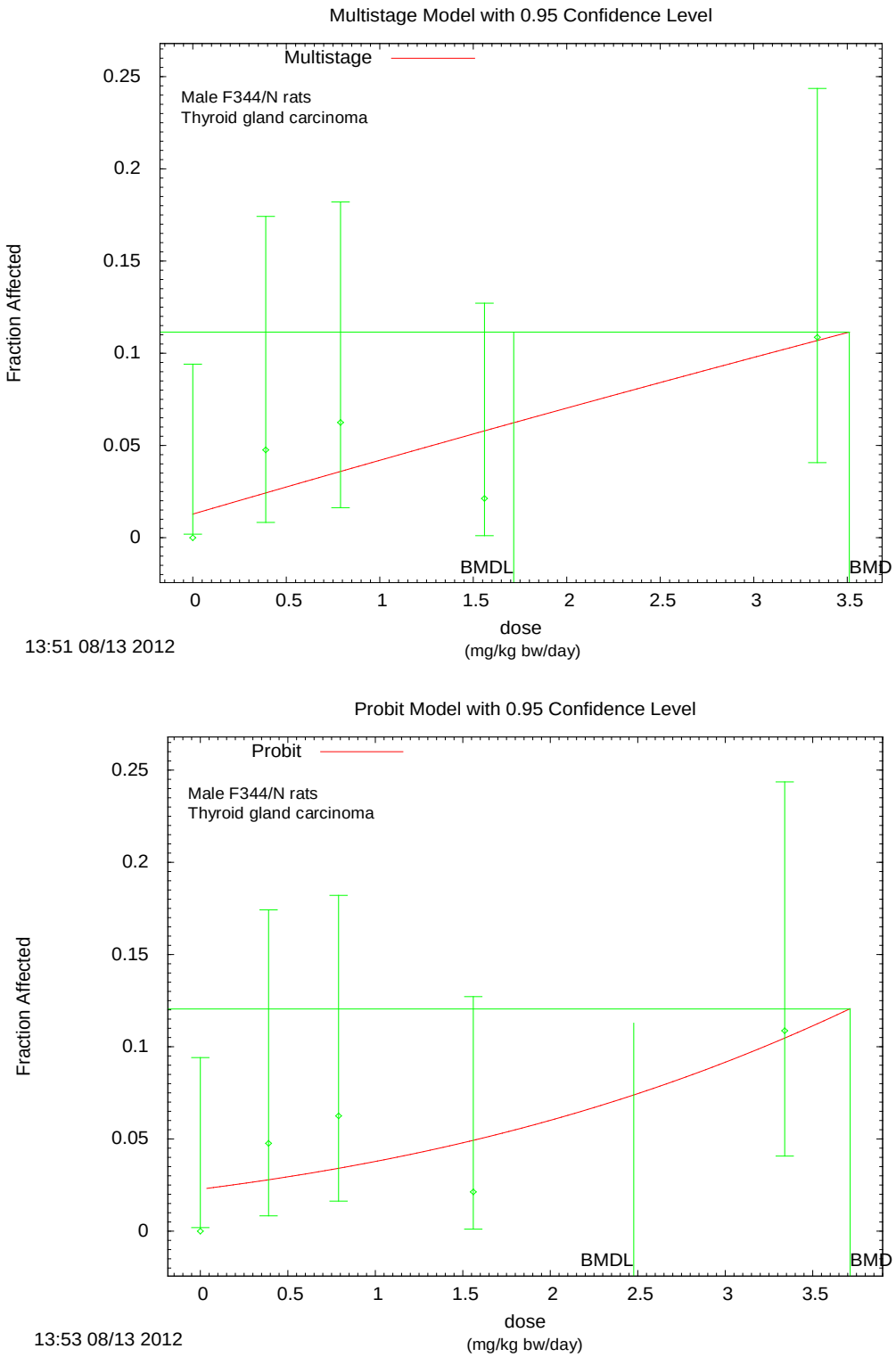


Figure K-3. Benchmark Dose Modeling of Thyroid Gland Carcinoma in Male F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Multistage Model; Bottom: Probit Model)

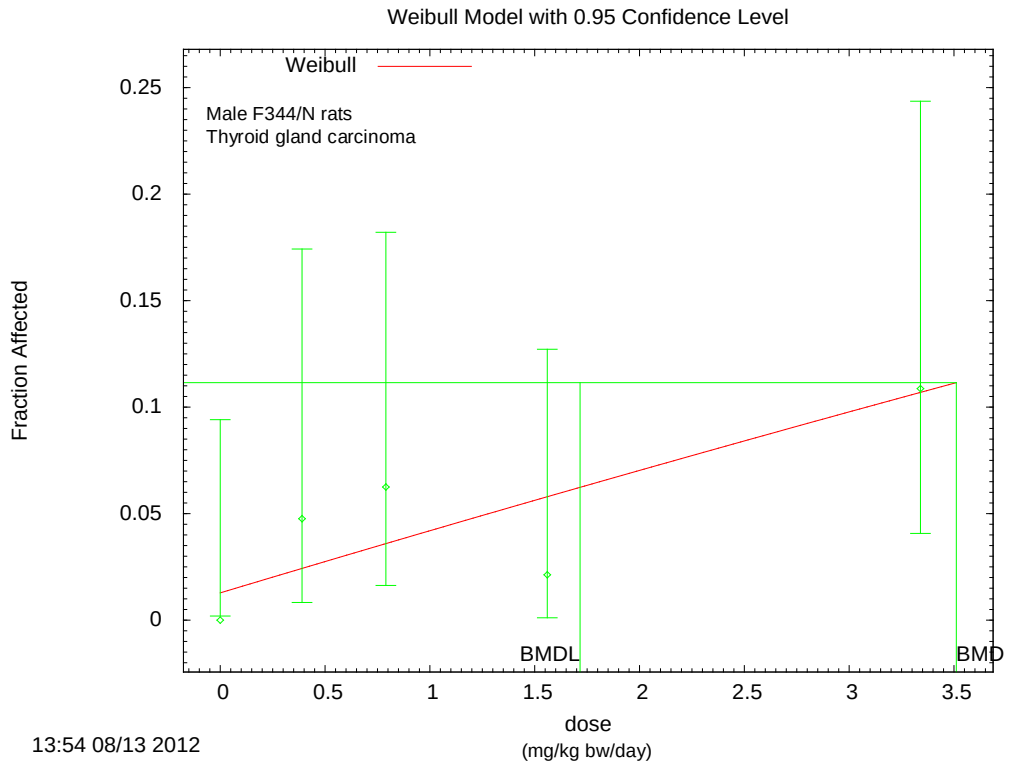


Figure K-4. Benchmark Dose Modeling of Thyroid Gland Carcinoma in Male F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Weibull Model)

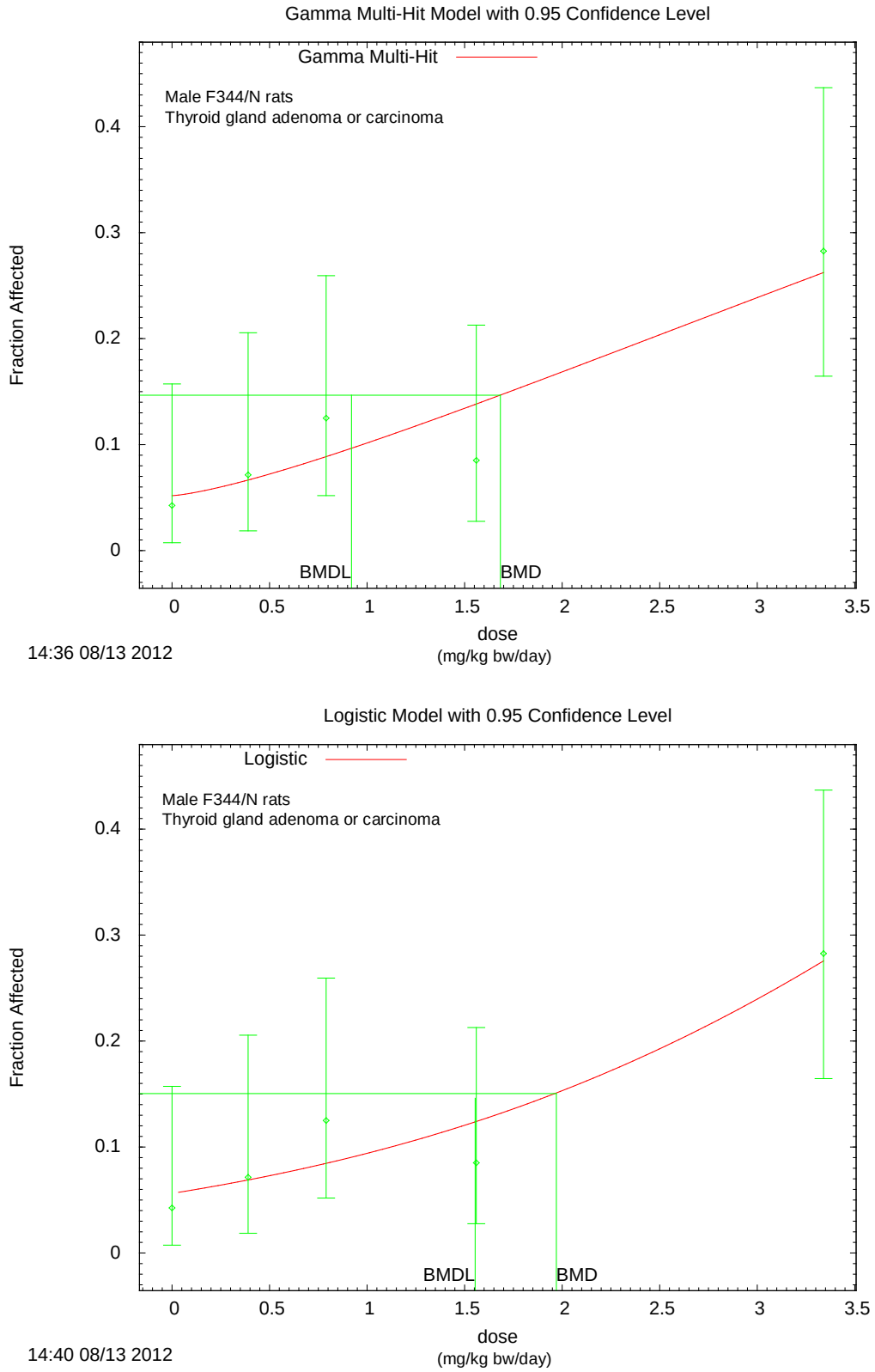


Figure K-5. Benchmark Dose Modeling of Thyroid Gland Adenoma or Carcinoma in Male F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Gamma Multi-hit Model; Bottom: Logistic Model)

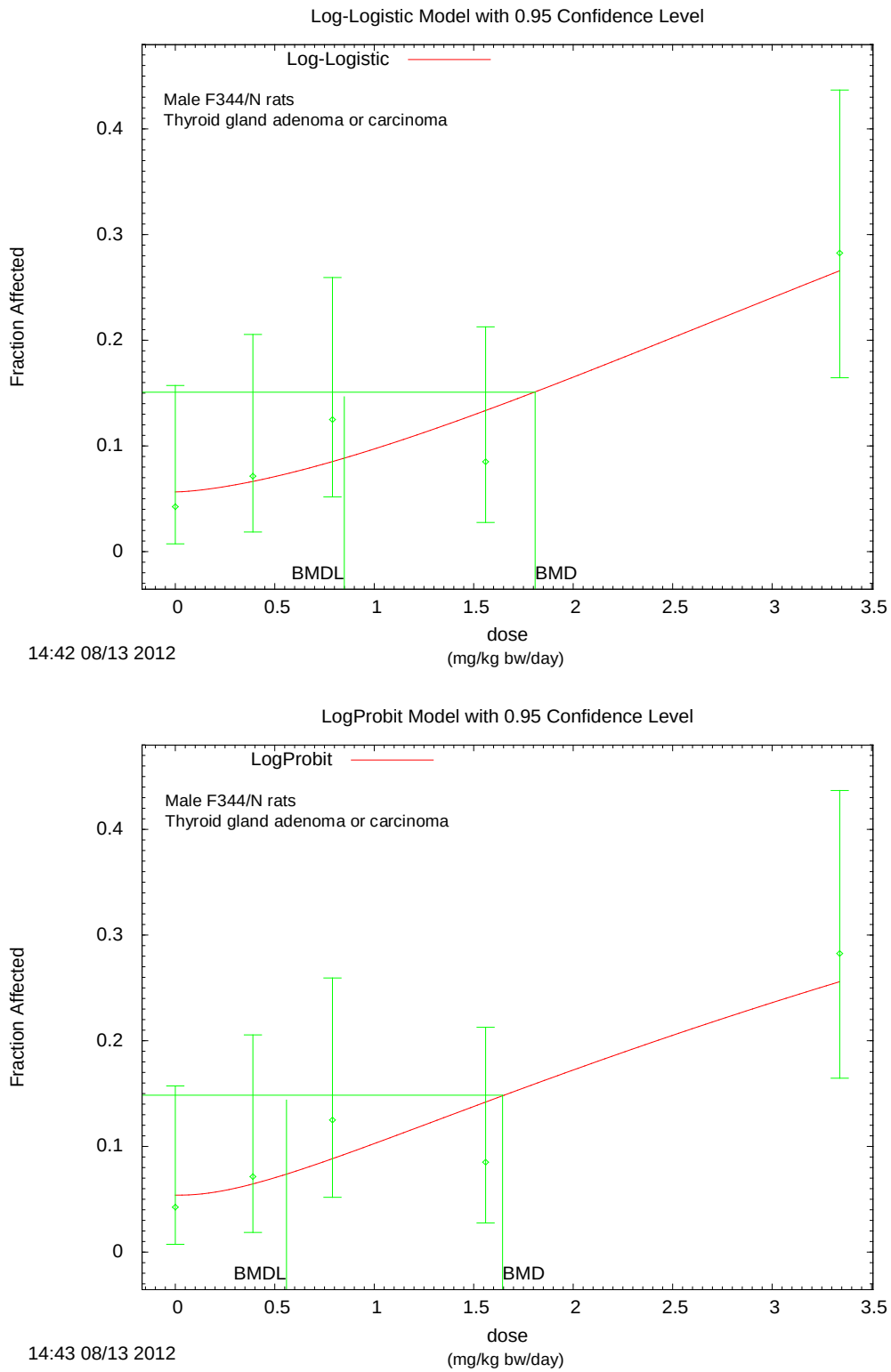


Figure K-6. Benchmark Dose Modeling of Thyroid Gland Adenoma or Carcinoma in Male F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Log-Logistic Model; Bottom: LogProbit Model)

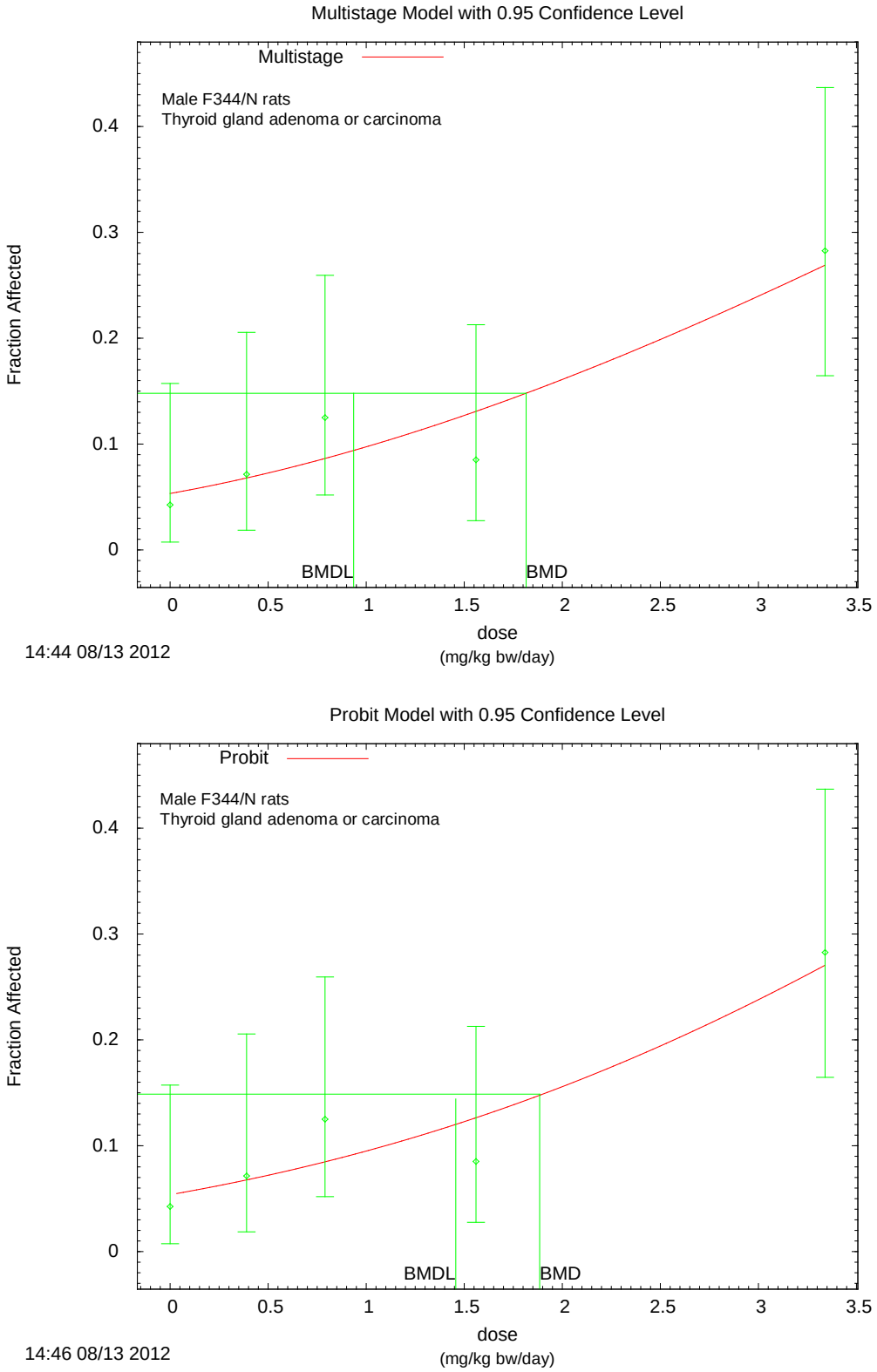


Figure K-7. Benchmark Dose Modeling of Thyroid Gland Adenoma or Carcinoma in Male F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Multistage Model; Bottom: Probit Model)

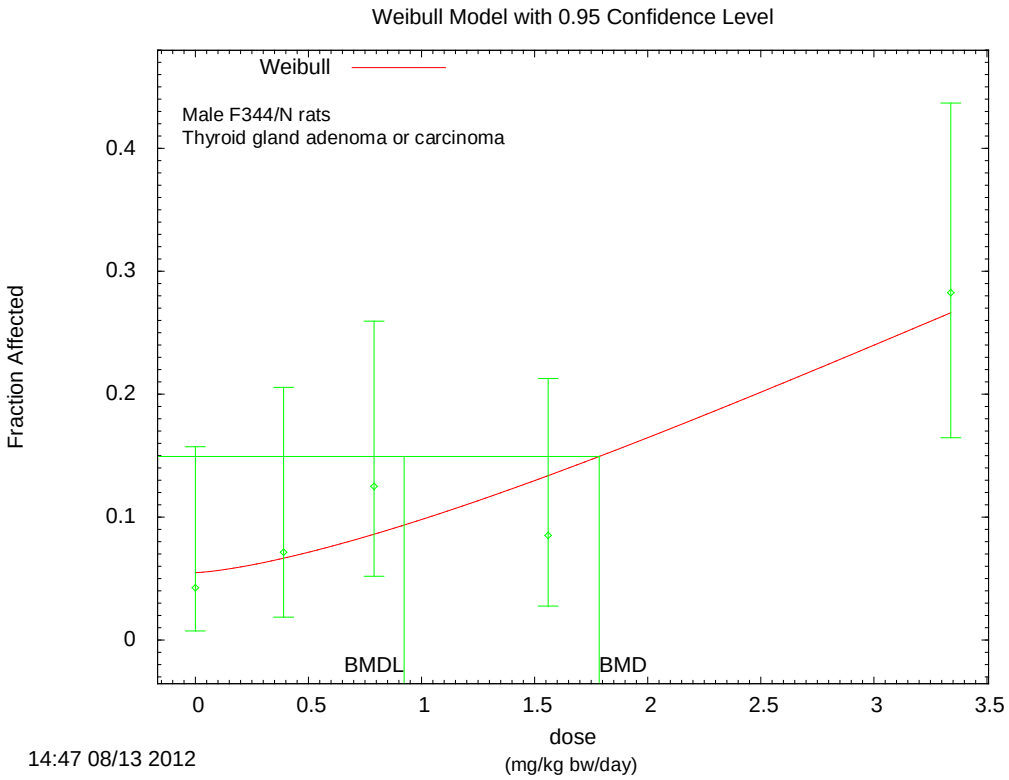


Figure K-8. Benchmark Dose Modeling of Thyroid Gland Adenoma or Carcinoma in Male F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Weibull Model)

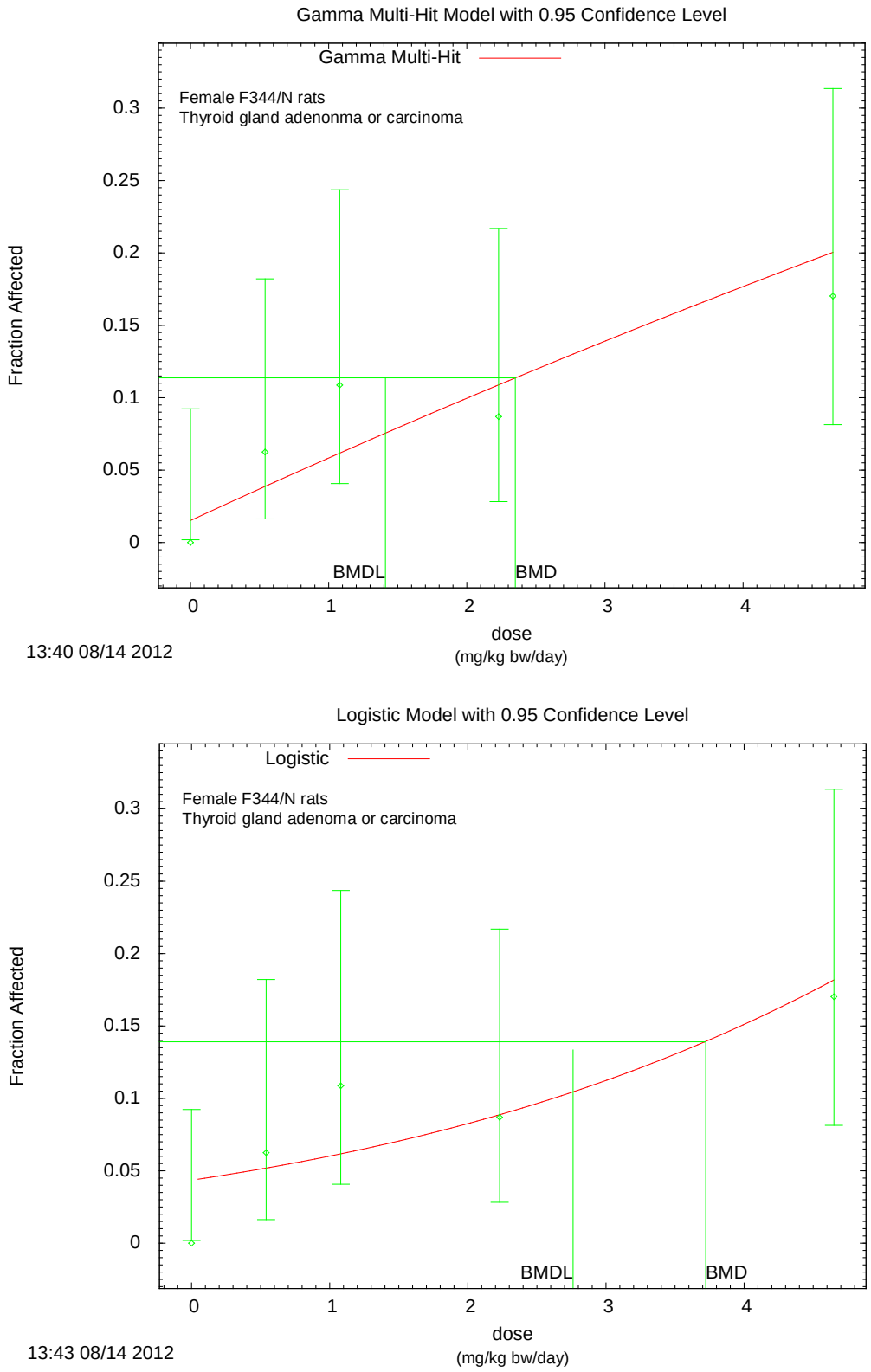


Figure K-9. Benchmark Dose Modeling of Thyroid Gland Adenoma or Carcinoma in Female F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Gamma Multi-hit Model; Bottom: Logistic Model)

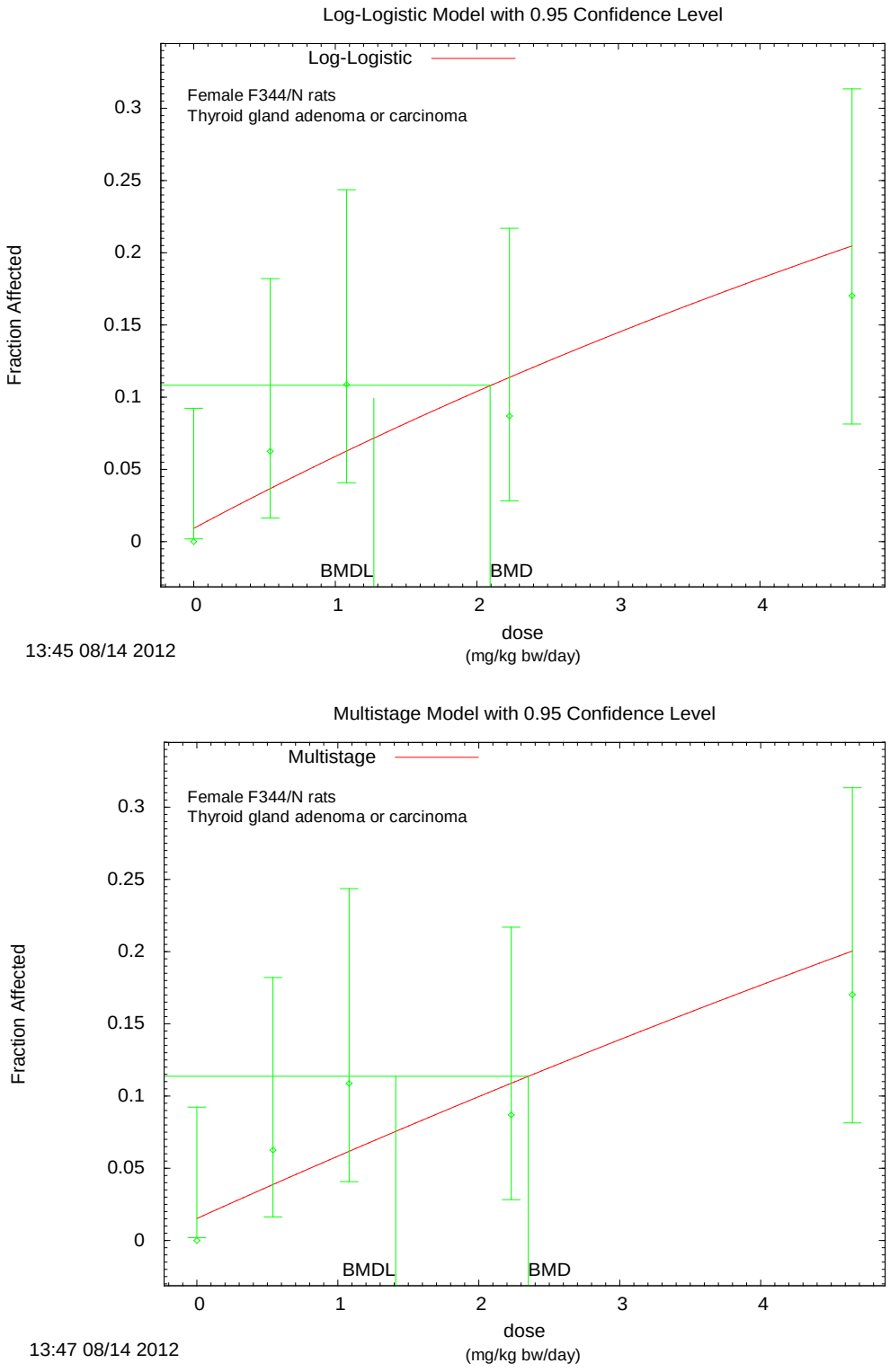


Figure K-10. Benchmark Dose Modeling of Thyroid Gland Adenoma or Carcinoma in Female F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Log-Logistic Model; Bottom: Multistage Model)

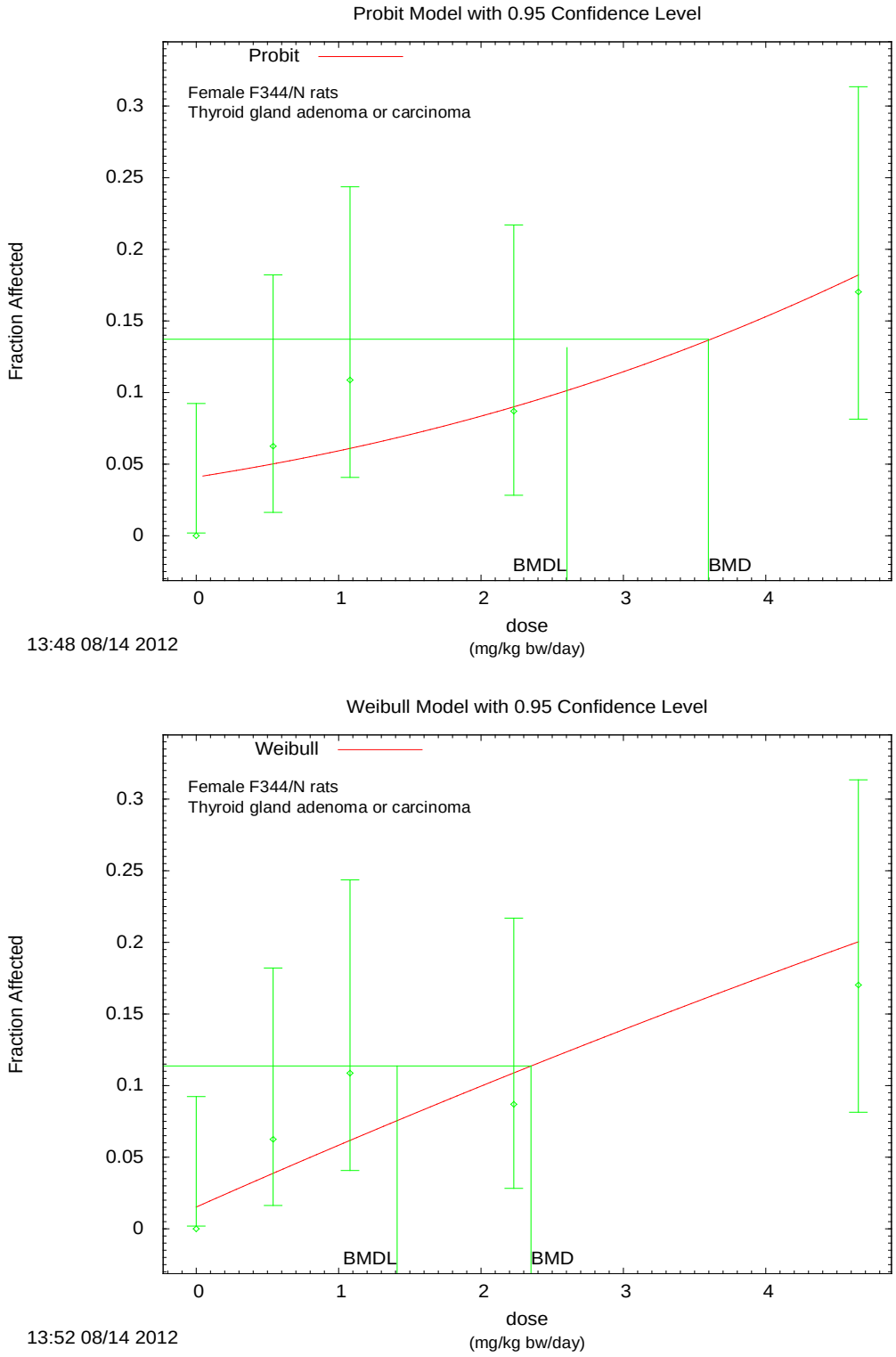


Figure K-11. Benchmark Dose Modeling of Thyroid Gland Adenoma or Carcinoma in Female F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Probit Model; Bottom: Weibull Model)

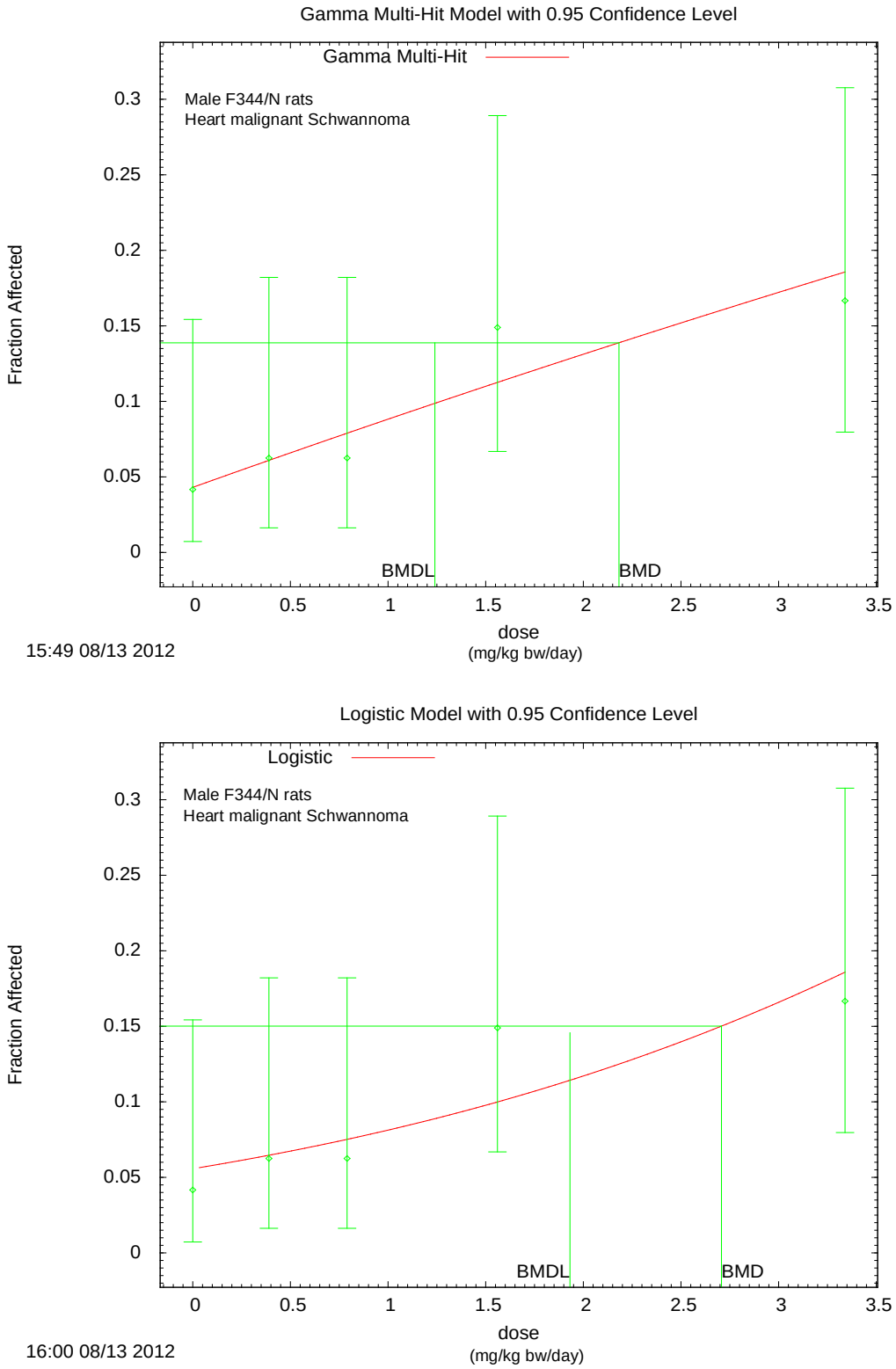


Figure K-12. Benchmark Dose Modeling of Heart Malignant Schwannoma in Male F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Gamma Multi-hit Model; Bottom: Logistic Model)

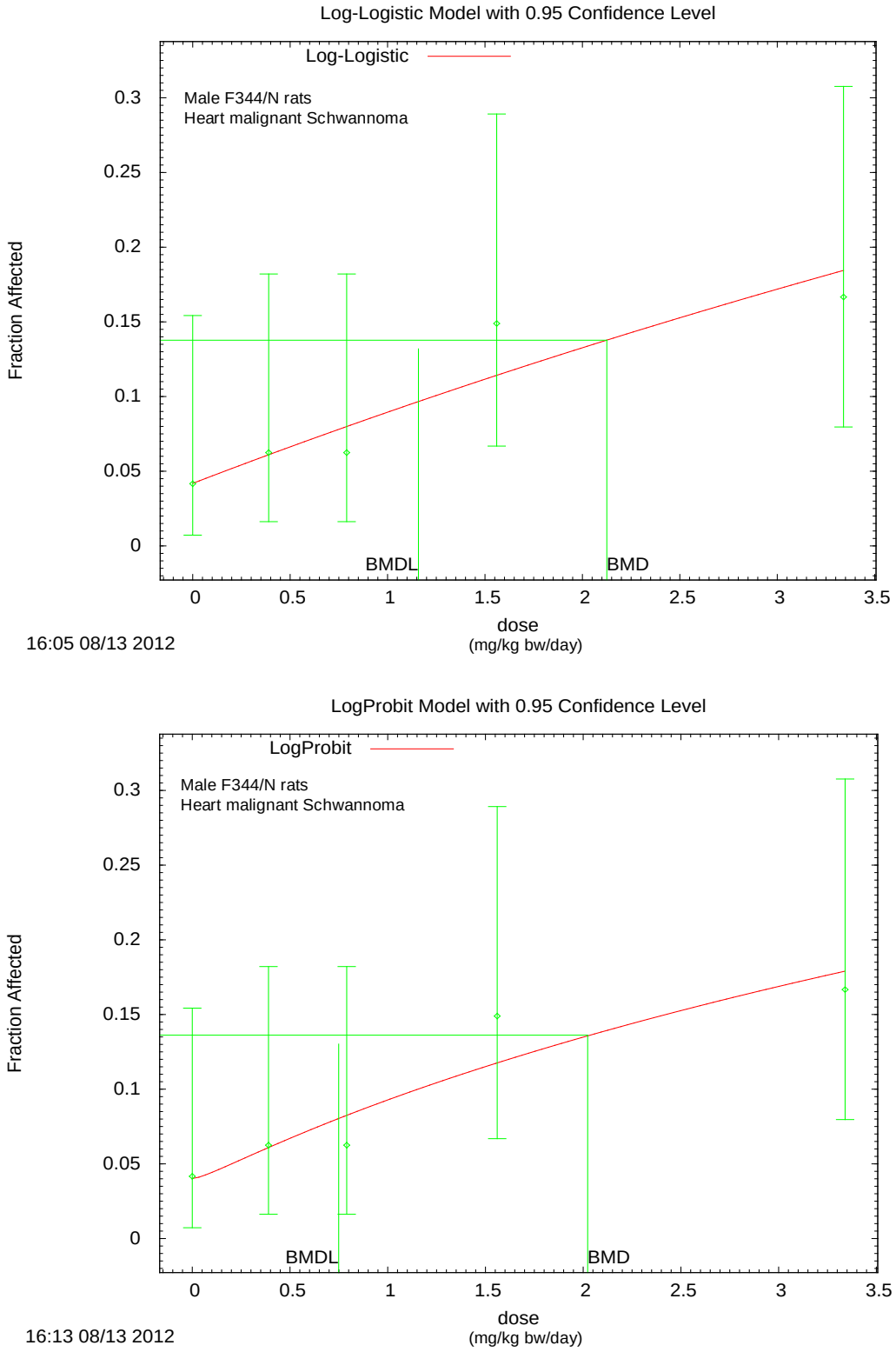


Figure K-13. Benchmark Dose Modeling of Heart Malignant Schwannoma in Male F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Log-Logistic Model; Bottom: LogProbit Model)

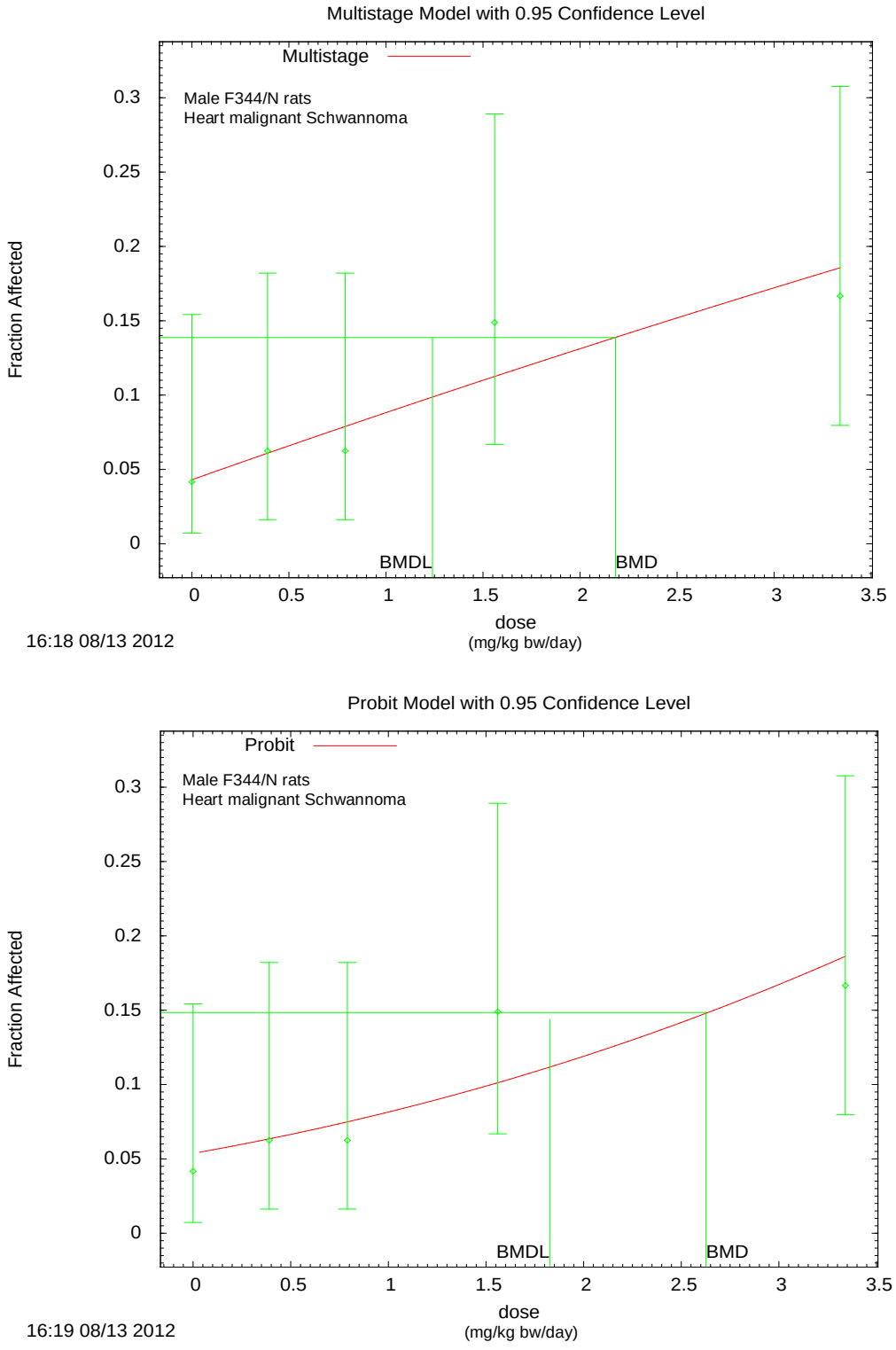


Figure K-14. Benchmark Dose Modeling of Heart Malignant Schwannoma in Male F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Multistage Model; Bottom: Probit Model)

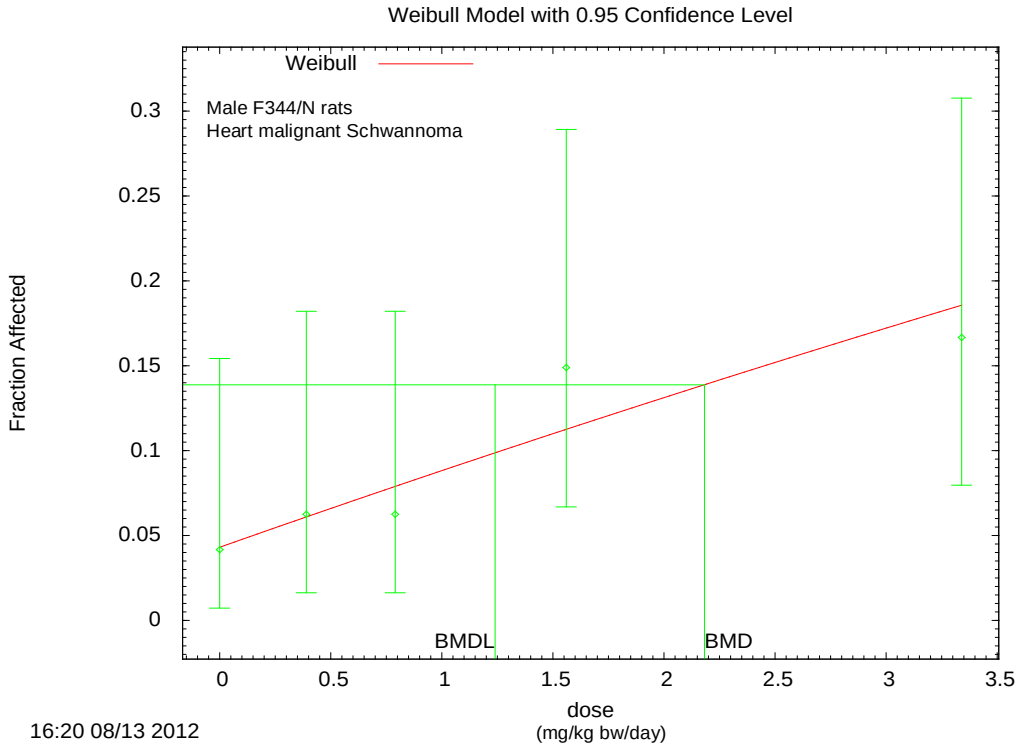
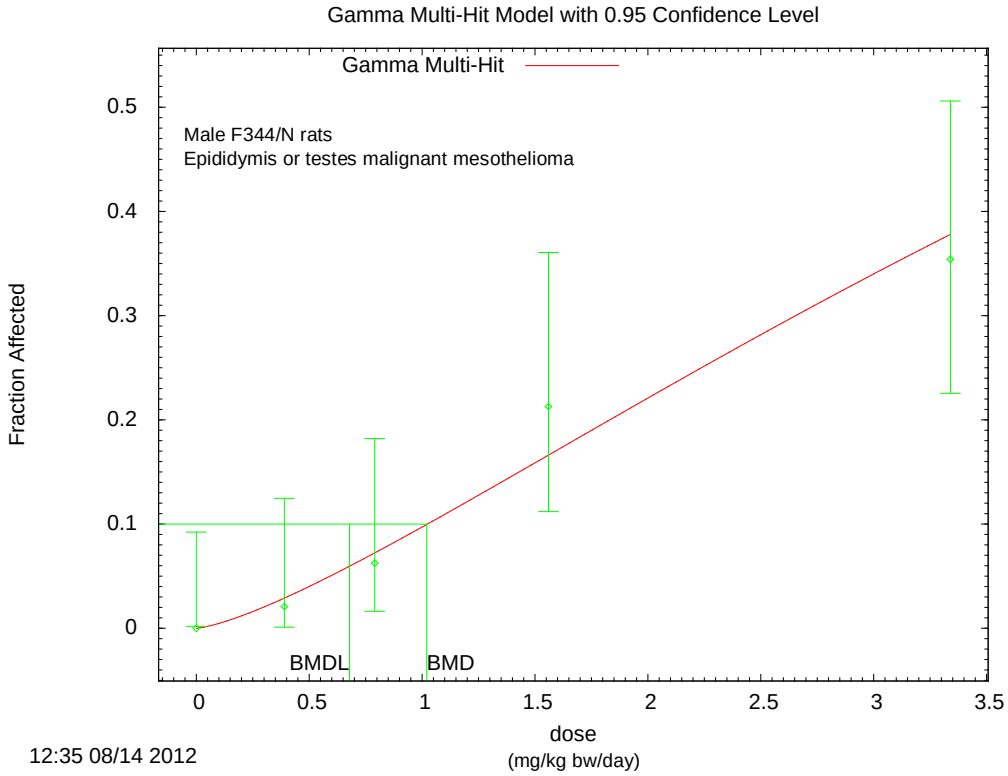


Figure K-15. Benchmark Dose Modeling of Heart Malignant Schwannoma in Male F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Weibull Model)



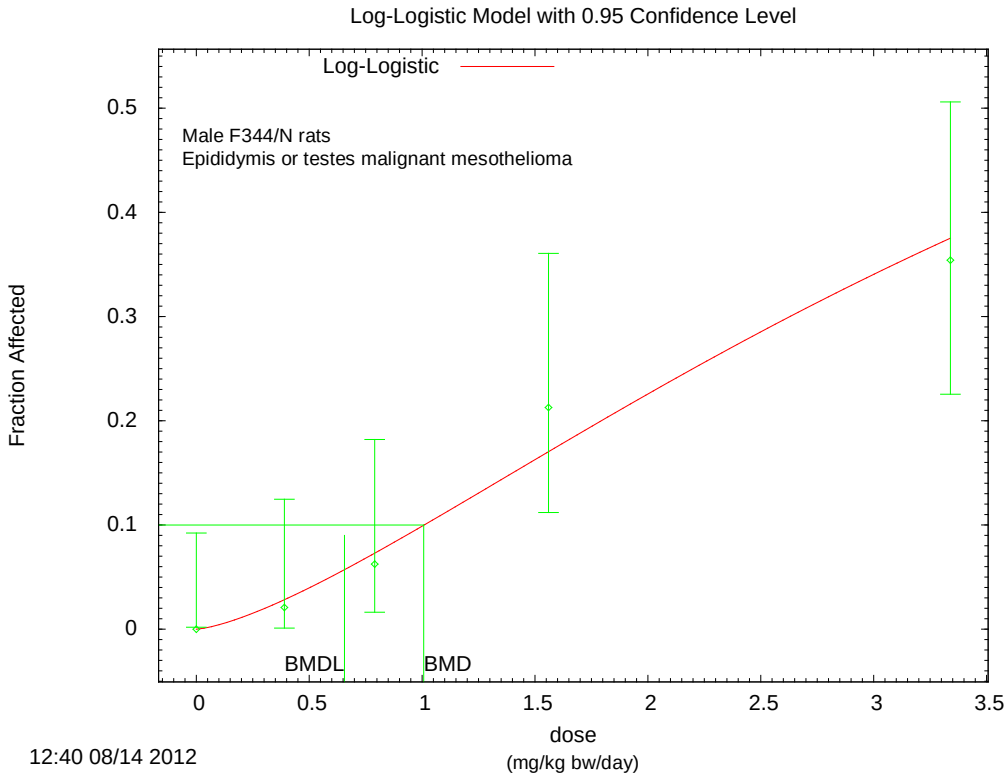


Figure K-16. Benchmark Dose Modeling of Epididymis or Testes Malignant Mesothelioma in Male F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Gamma Multi-hit Model; Bottom: Log-Logistic Model)

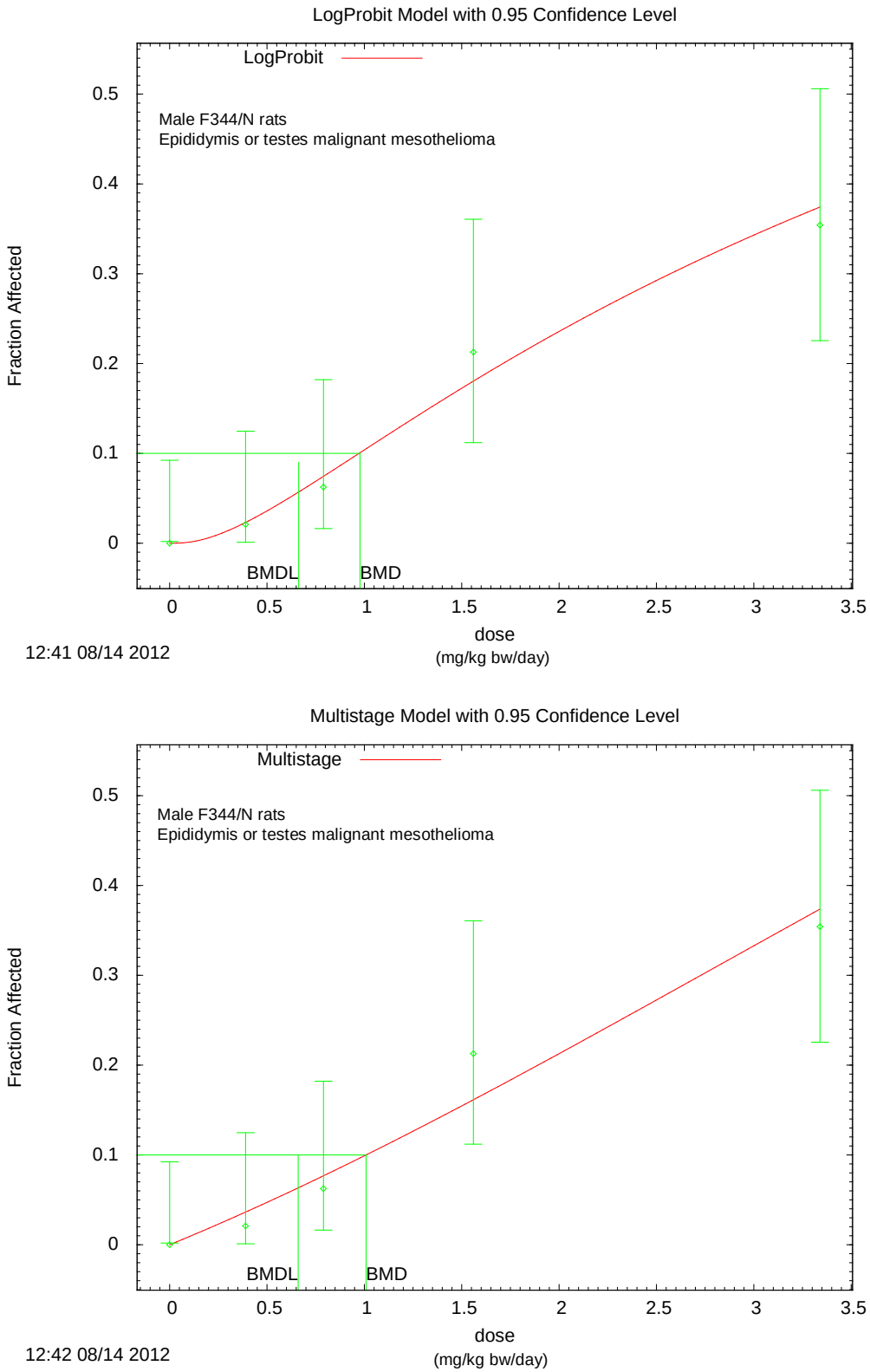


Figure K-17. Benchmark Dose Modeling of Epididymis or Testes Malignant Mesothelioma in Male F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: LogProbit Model; Bottom: Multistage Model)

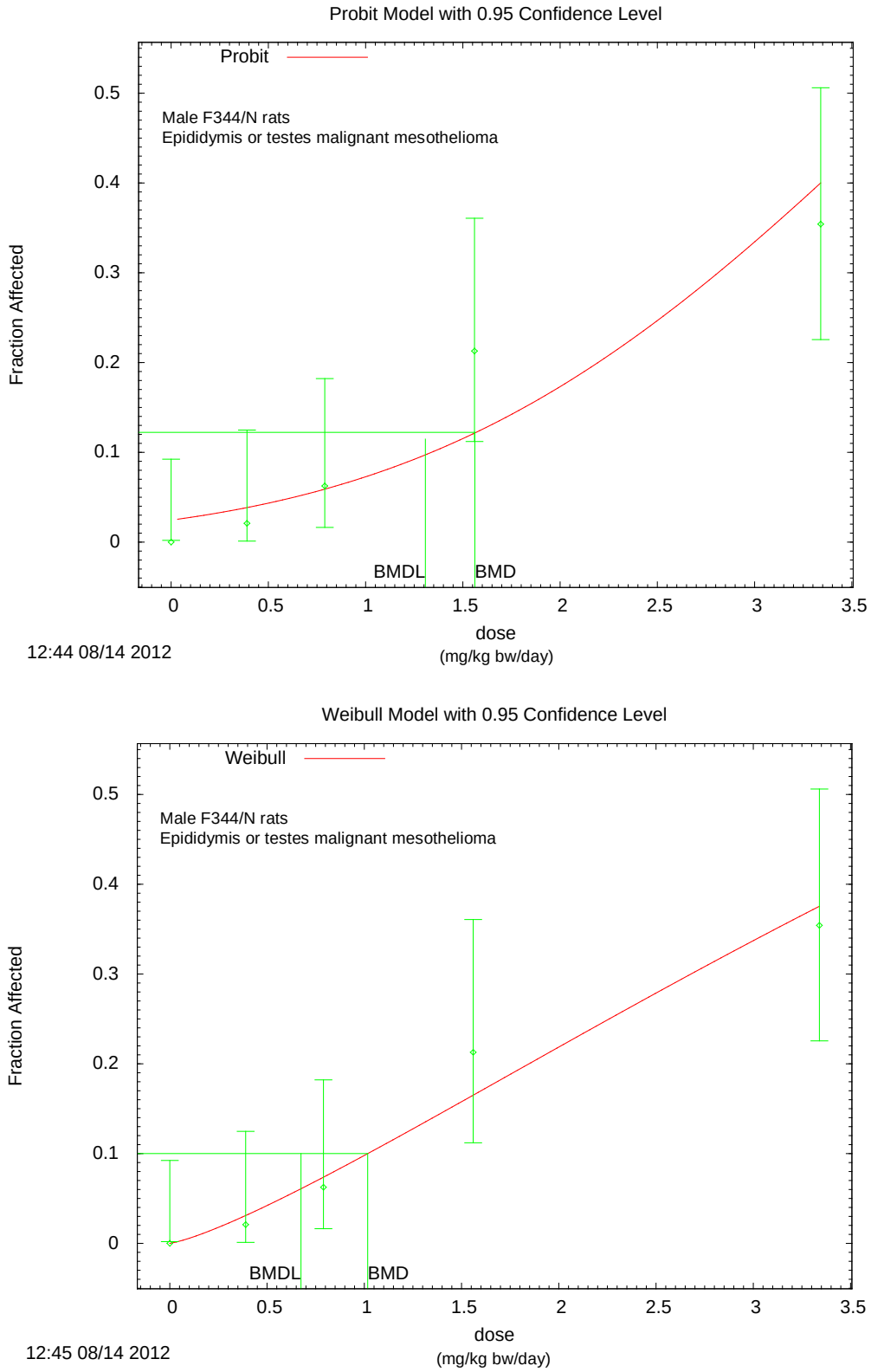


Figure K-18. Benchmark Dose Modeling of Epididymis or Testes Malignant Mesothelioma in Male F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Probit Model; Bottom: Weibull Model)

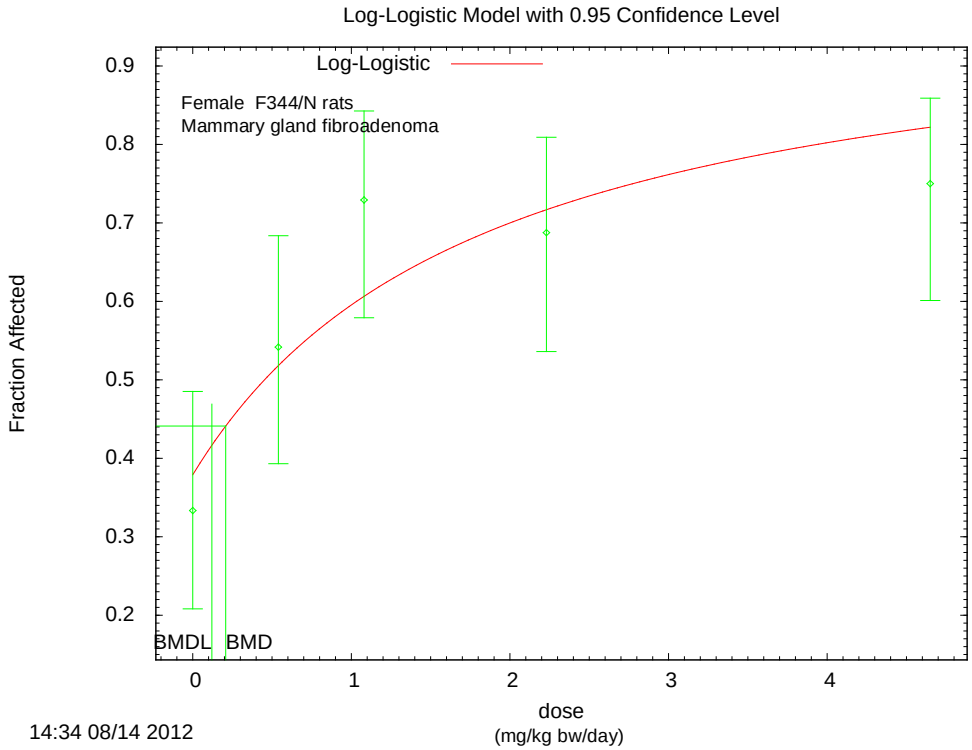
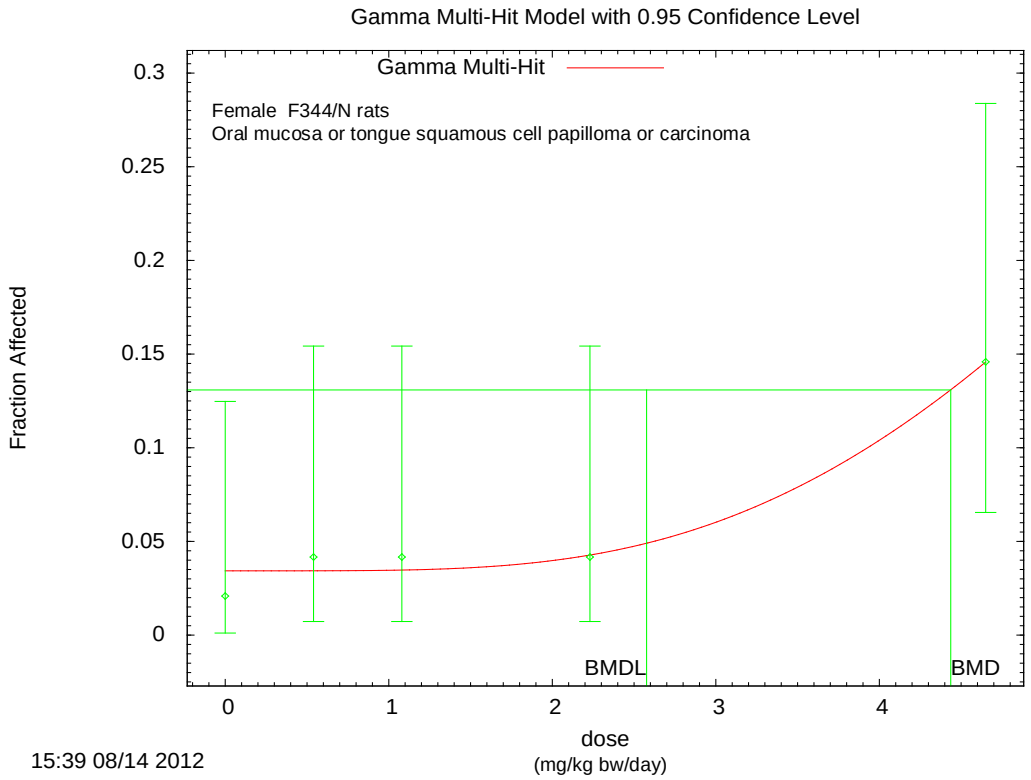


Figure K-19. Benchmark Dose Modeling of Mammary Gland Fibroadenoma in Female F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Log-Logistic Model)



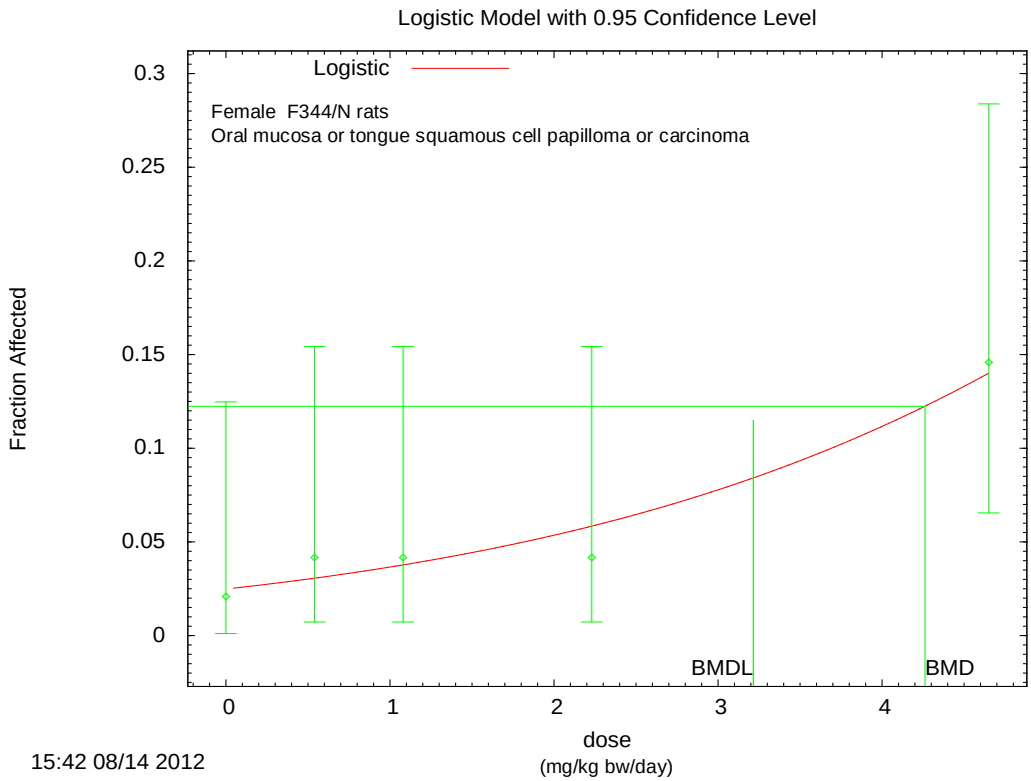


Figure K-20. Benchmark Dose Modeling of Oral Mucosa or Tongue Squamous Cell Papilloma or Carcinoma in Female F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Gamma Multi-hit Model; Bottom: Logistic Model)

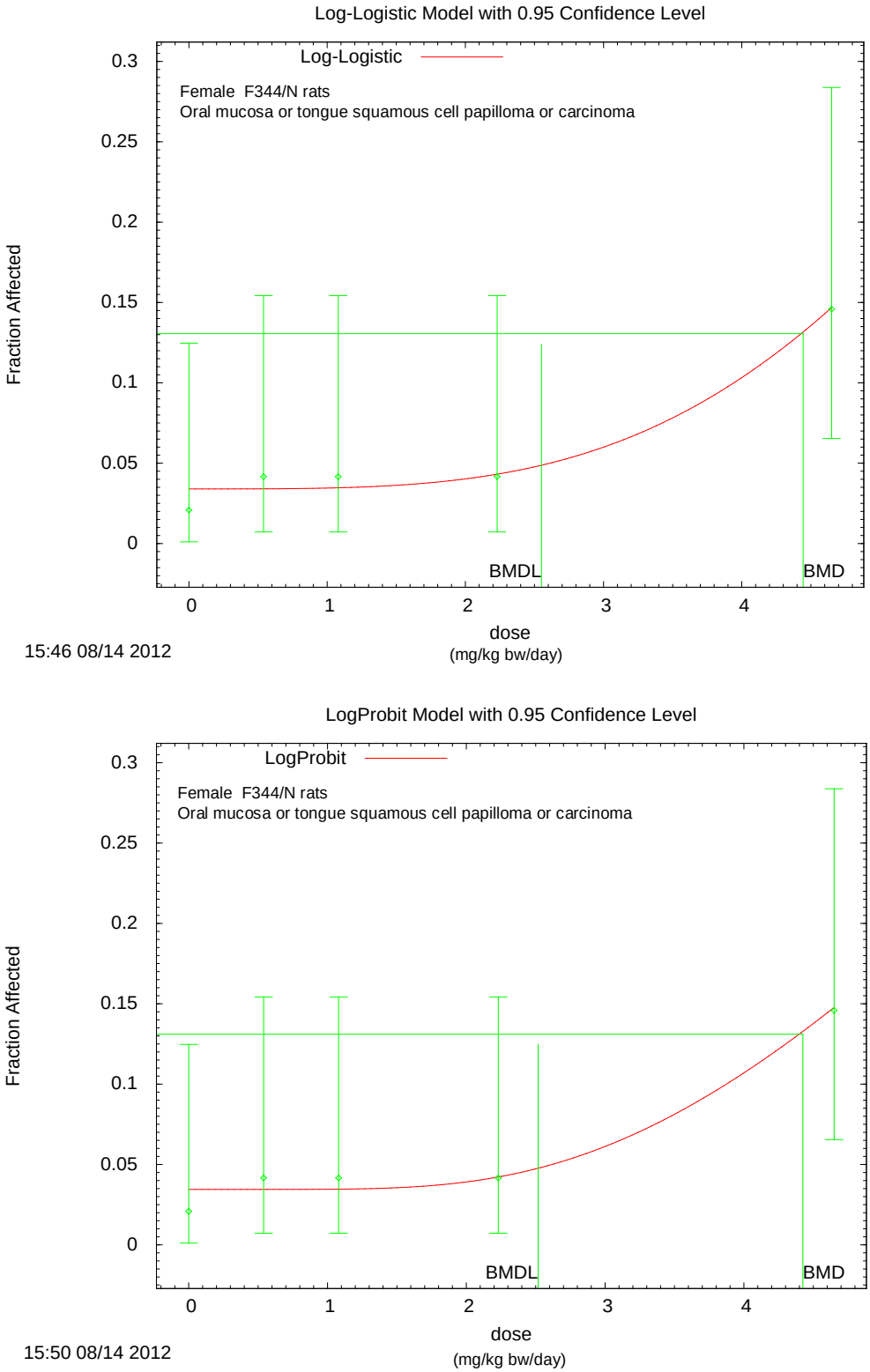
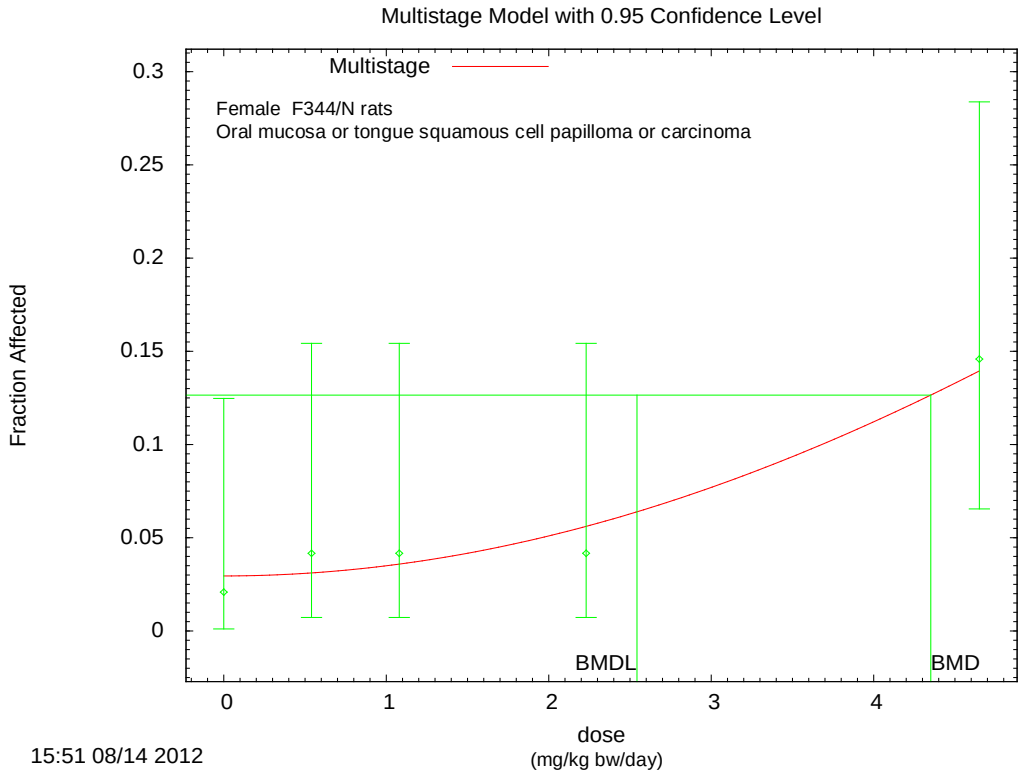


Figure K-21. Benchmark Dose Modeling of Oral Mucosa or Tongue Squamous Cell Papilloma or Carcinoma in Female F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Log-Logistic Model; Bottom: LogProbit Model)



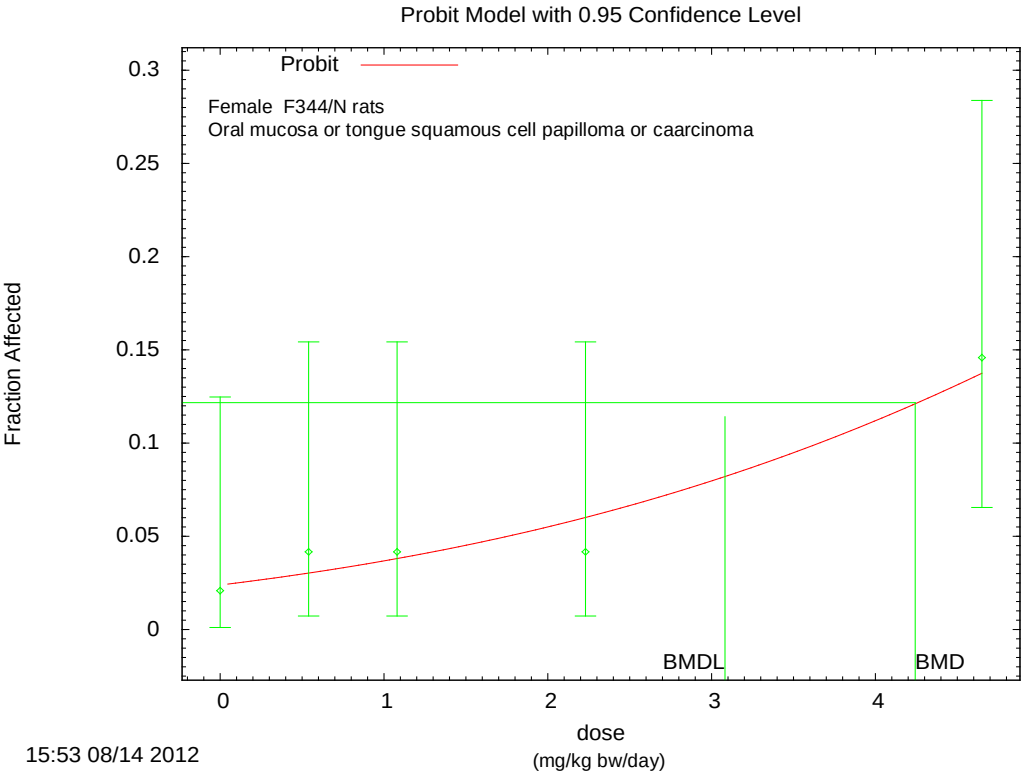


Figure K-22. Benchmark Dose Modeling of Oral Mucosa or Tongue Squamous Cell Papilloma or Carcinoma in Female F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Top: Multistage Model; Bottom: Probit Model)

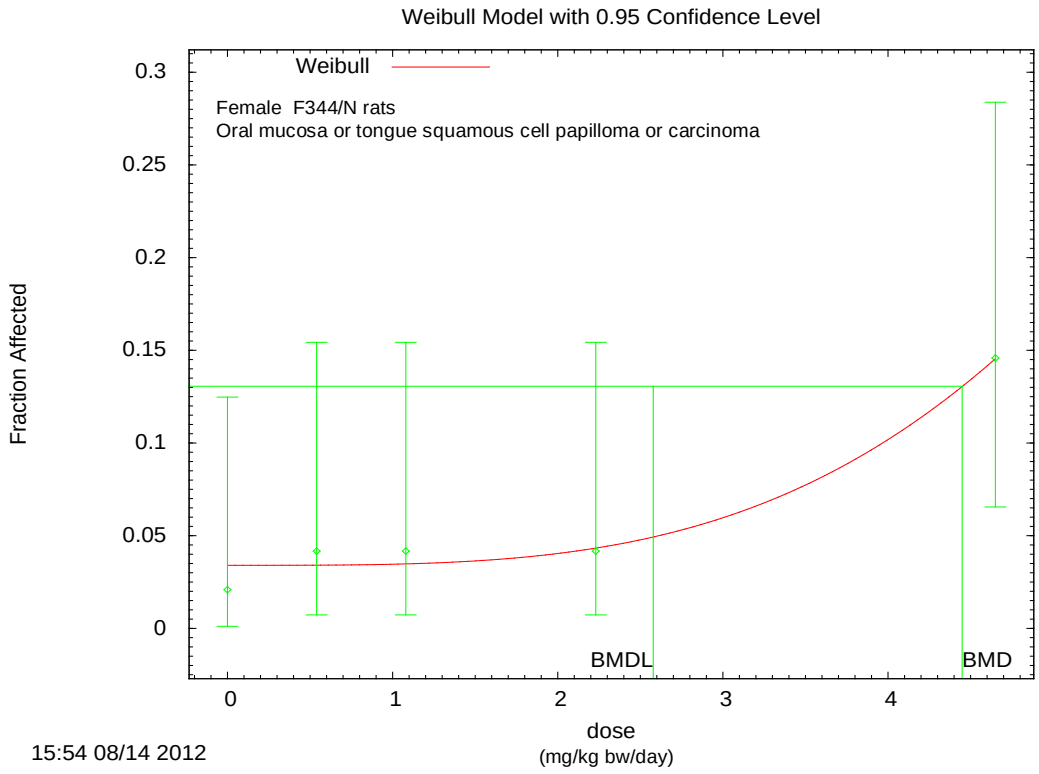


Figure K-23. Benchmark Dose Modeling of Oral Mucosa or Tongue Squamous Cell Papilloma or Carcinoma in Female F344/N Nctr Rats in the Two-year Drinking Water Study of Glycidamide (Weibull Model)

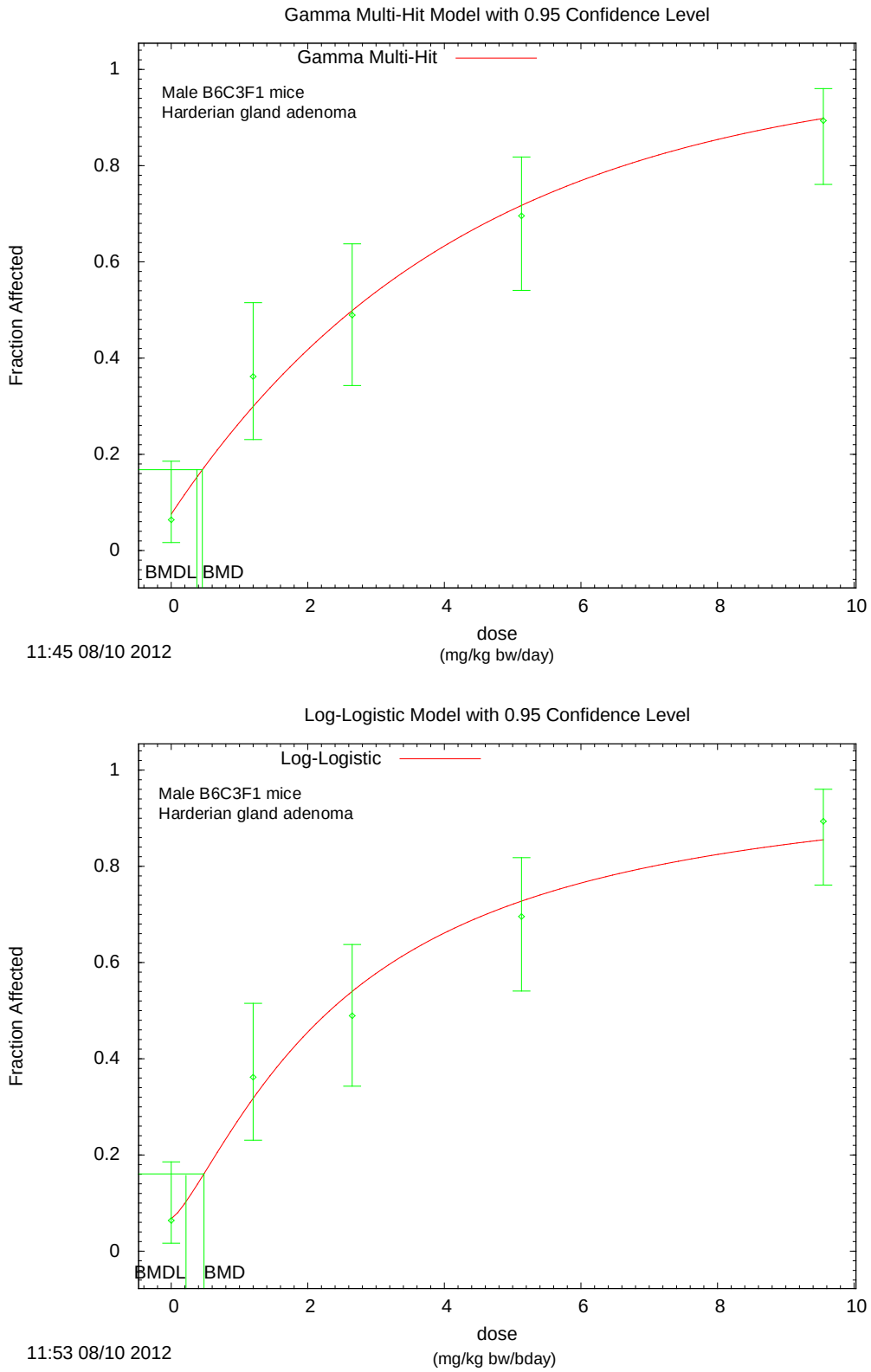


Figure K-24. Benchmark Dose Modeling of Harderian Gland Adenoma in Male B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: Gamma Multi-hit Model; Bottom: Log-Logistic Model)

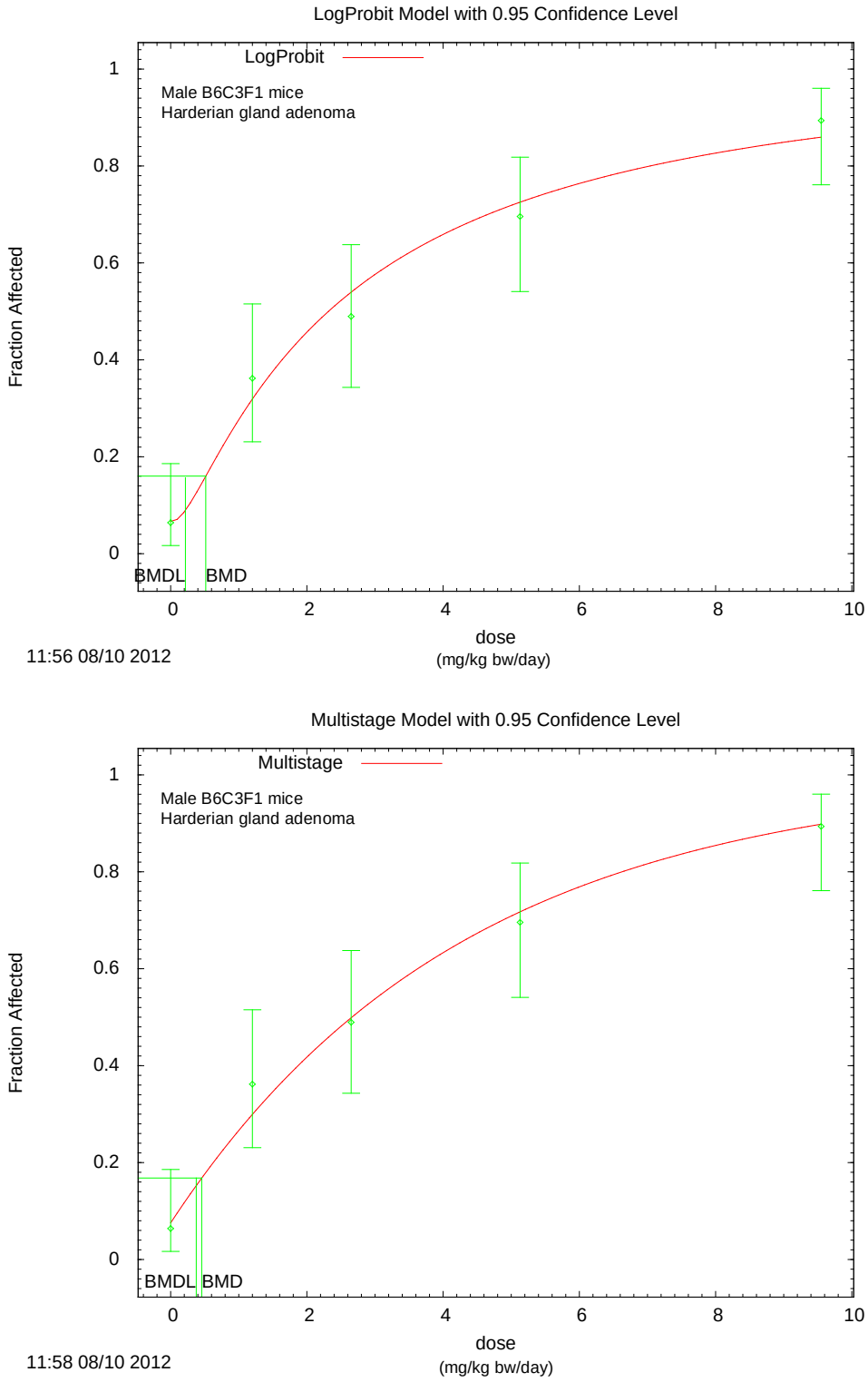


Figure K-25. Benchmark Dose Modeling of Harderian Gland Adenoma in Male B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: LogProbit Model; Bottom: Multistage Model)

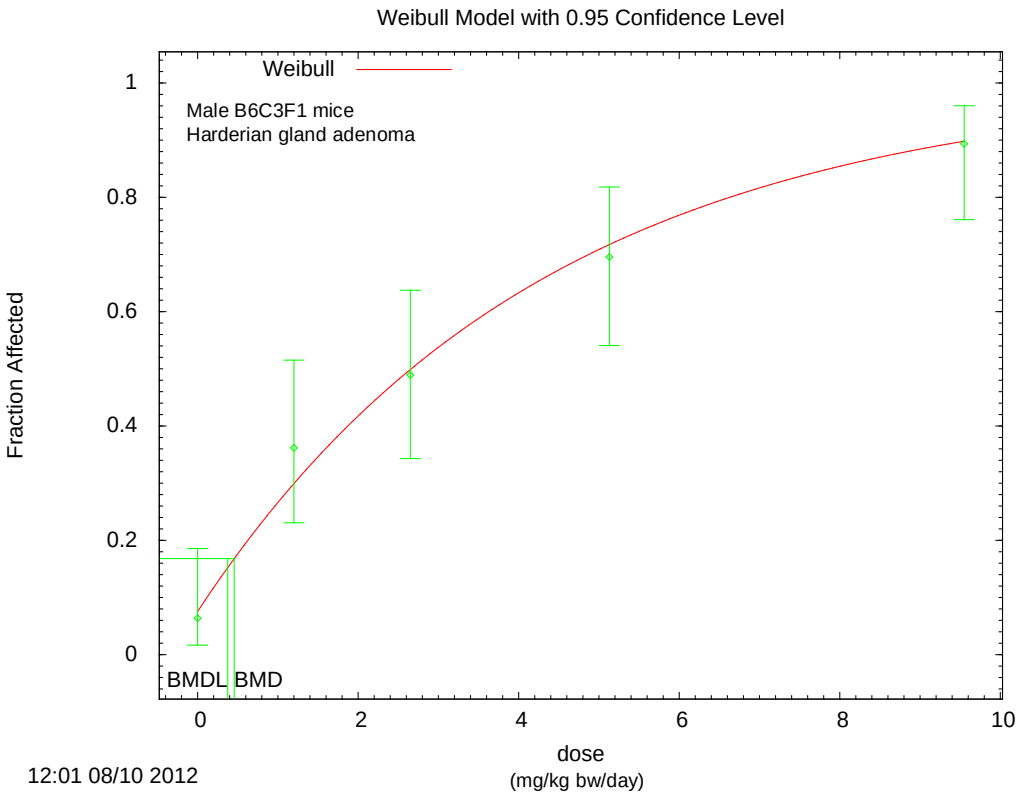


Figure K-26. Benchmark Dose Modeling of Harderian Gland Adenoma in Male B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Weibull Model)

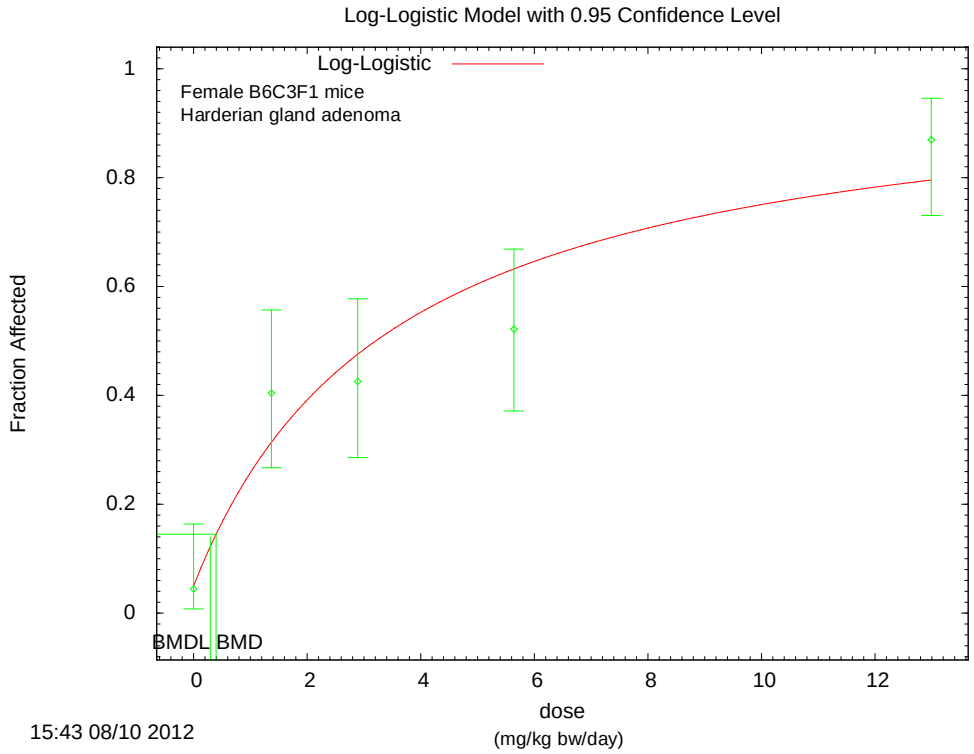


Figure K-27. Benchmark Dose Modeling of Harderian Gland Adenoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Log-Logistic Model)

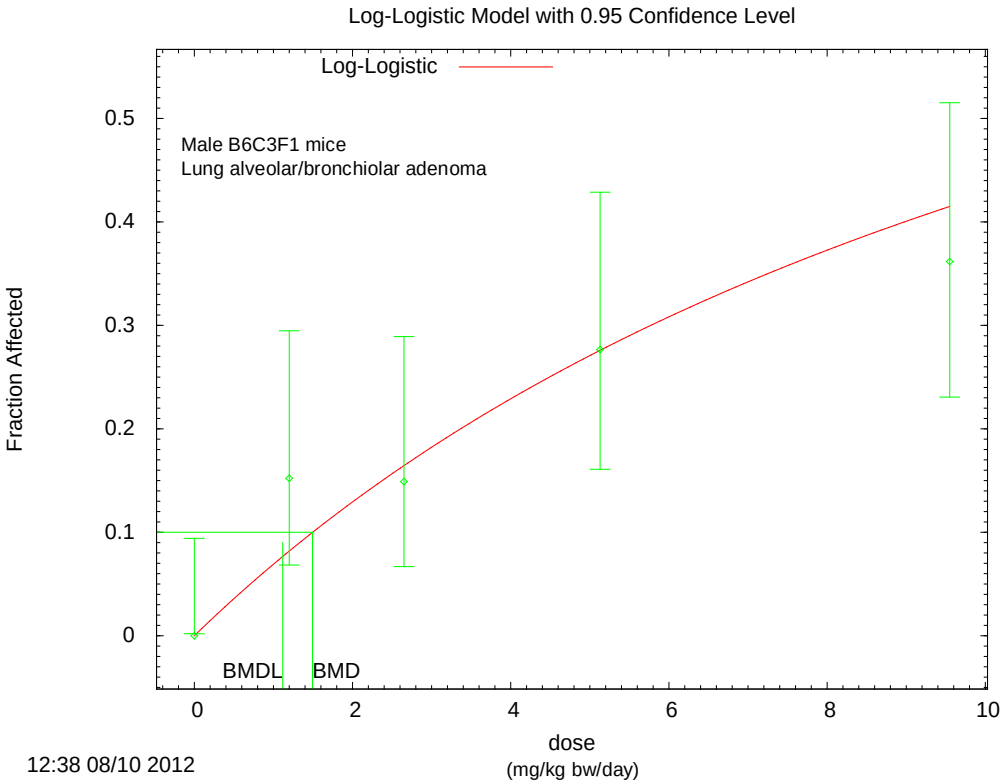


Figure K-28. Benchmark Dose Modeling of Lung Alveolar/Bronchiolar Adenoma in Male B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Log-Logistic Model)

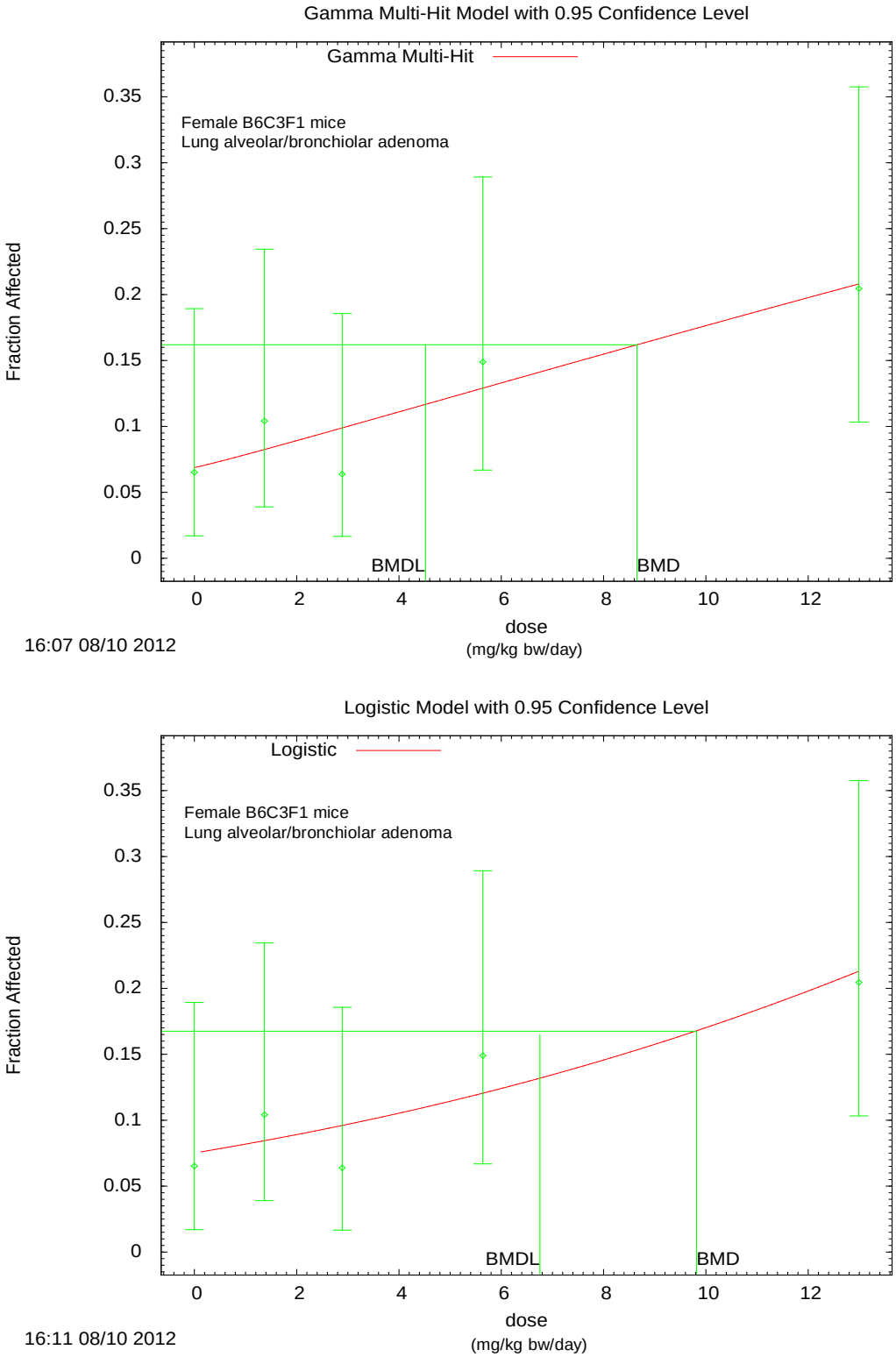


Figure K-29. Benchmark Dose Modeling of Lung Alveolar/Bronchiolar Adenoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: Gamma Multi-hit Model; Bottom: Logistic Model)

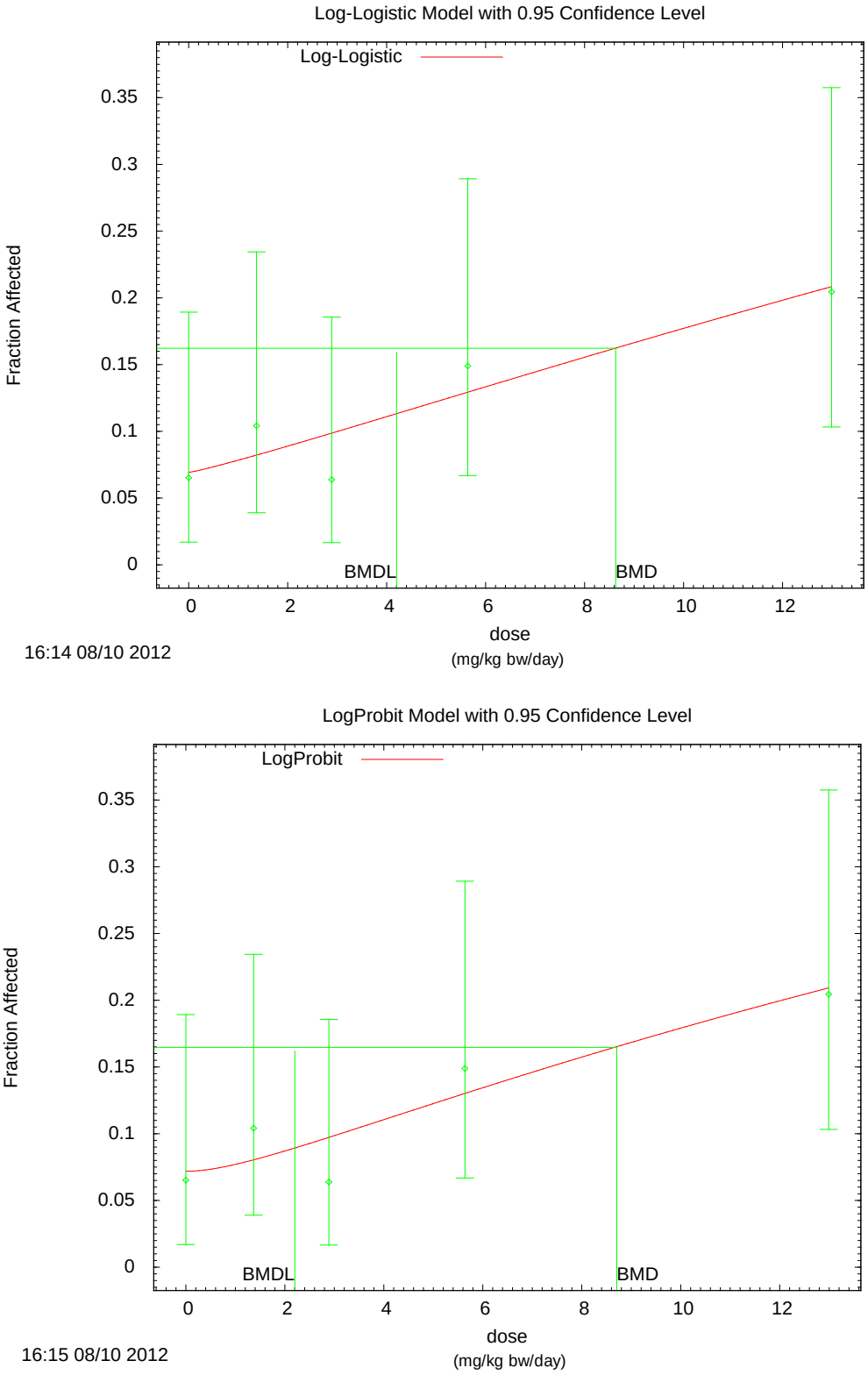
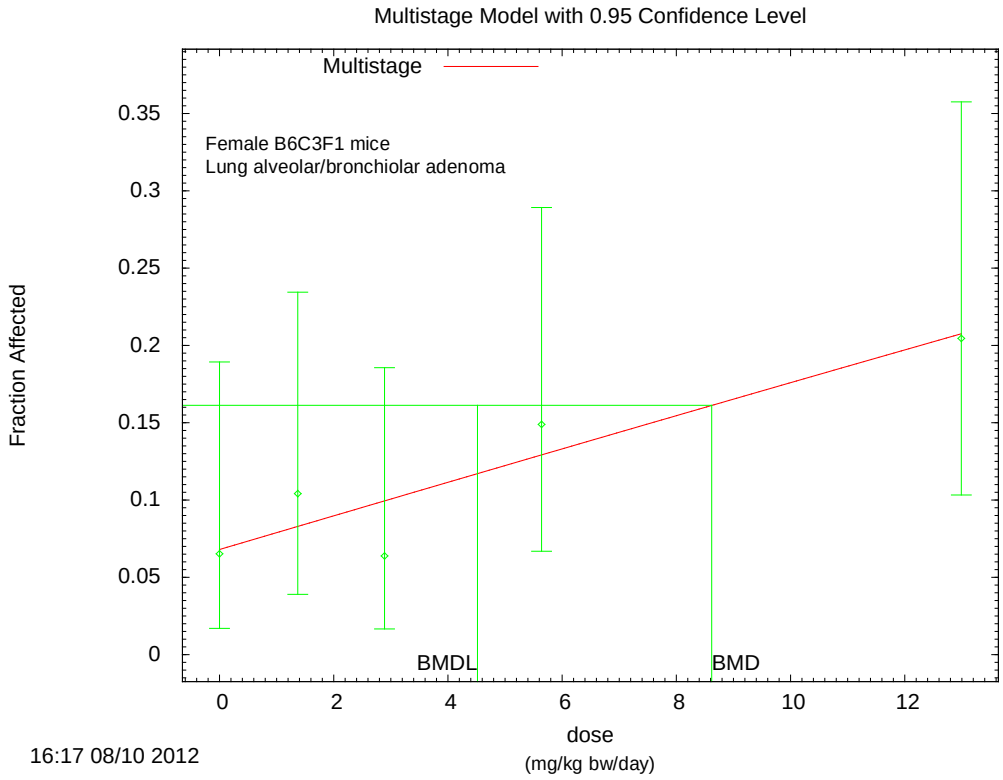


Figure K-30. Benchmark Dose Modeling of Lung Alveolar/Bronchiolar Adenoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: Log-Logistic Model; Bottom: LogProbit Model)



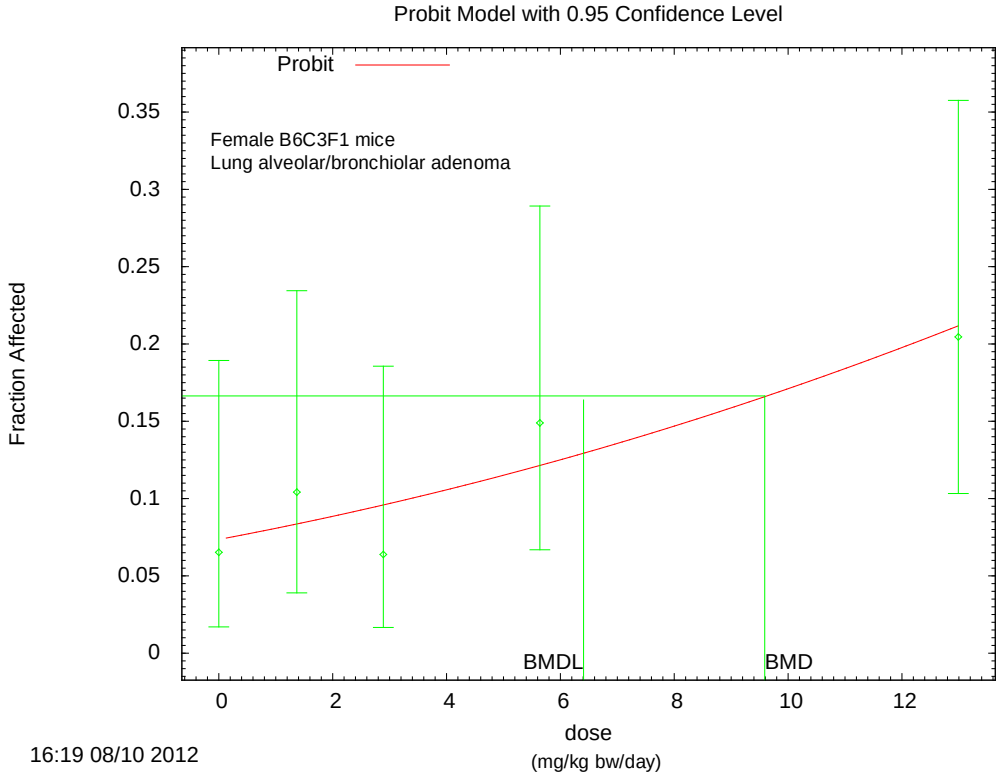


Figure K-31. Benchmark Dose Modeling of Lung Alveolar/Bronchiolar Adenoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: Multistage Model; Bottom: Probit Model)

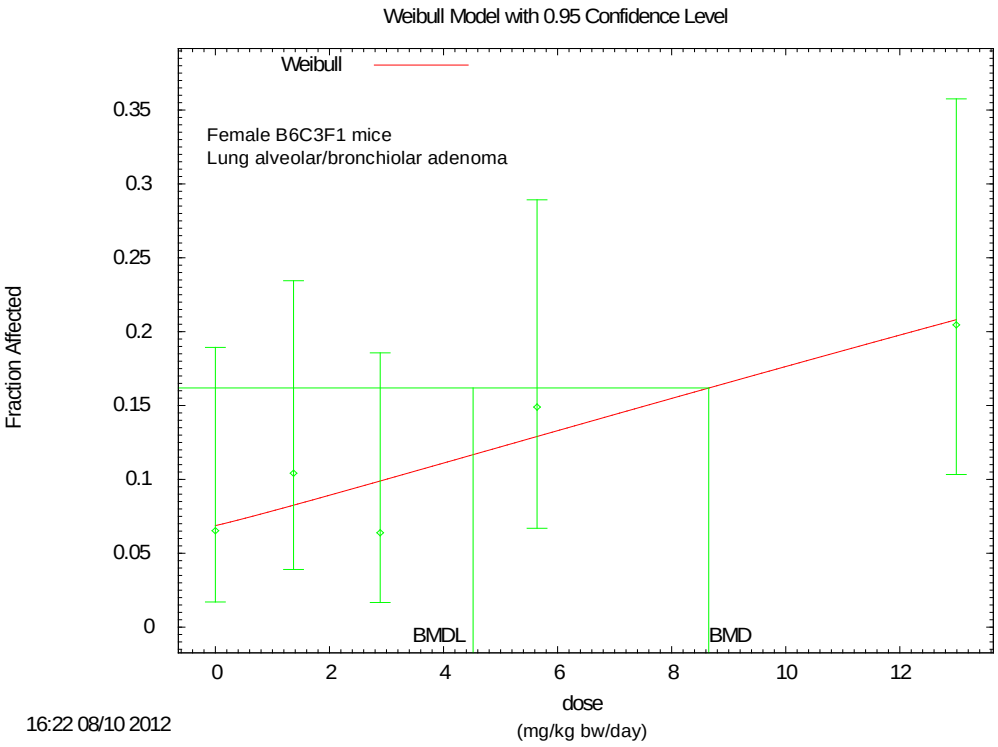


Figure K-32. Benchmark Dose Modeling of Lung Alveolar/Bronchiolar Adenoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Weibull Model)

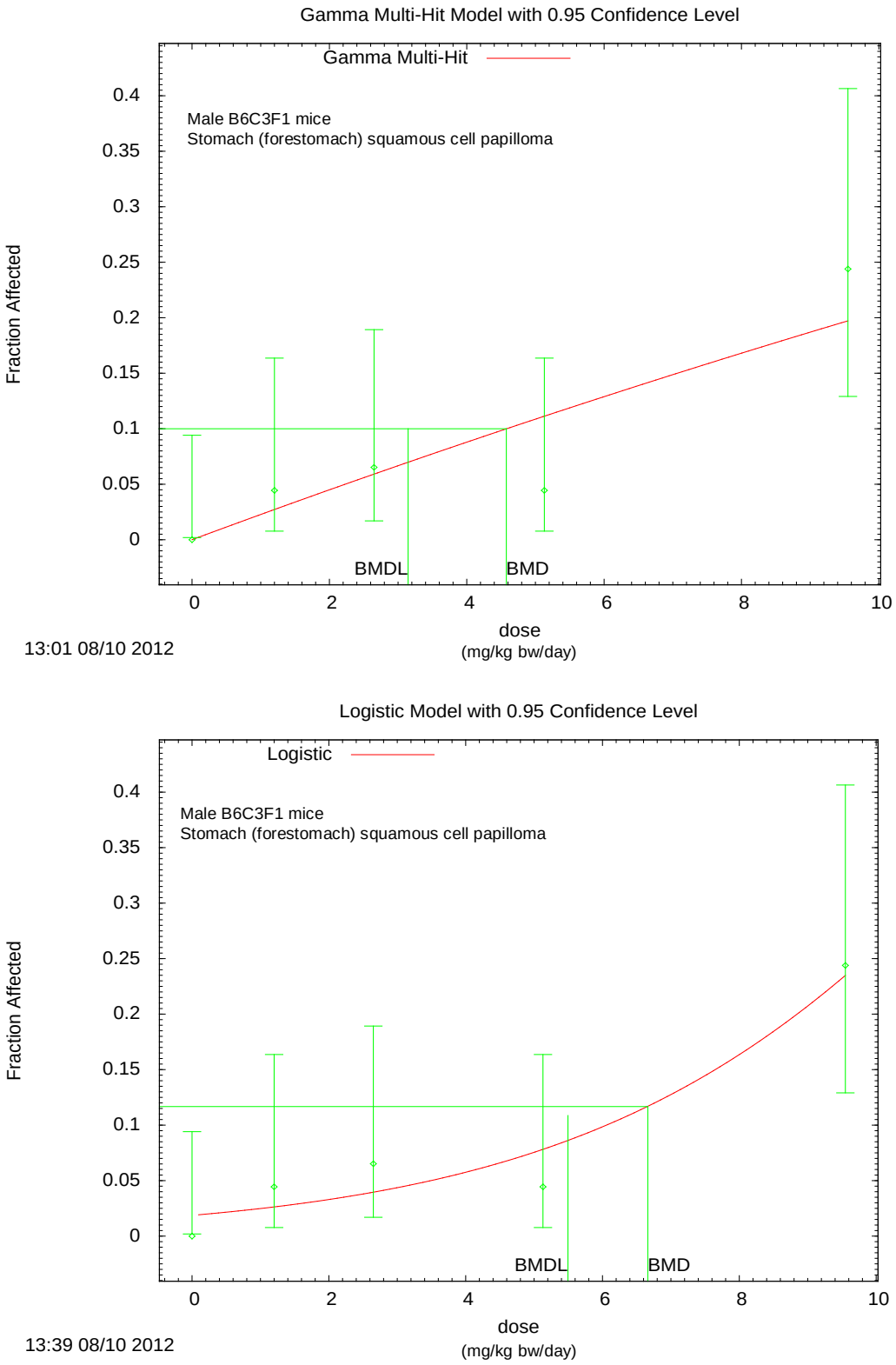


Figure K-33. Benchmark Dose Modeling of Stomach (Forestomach) Squamous Cell Papilloma in Male B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: Gamma Multi-hit Model; Bottom: Logistic Model)

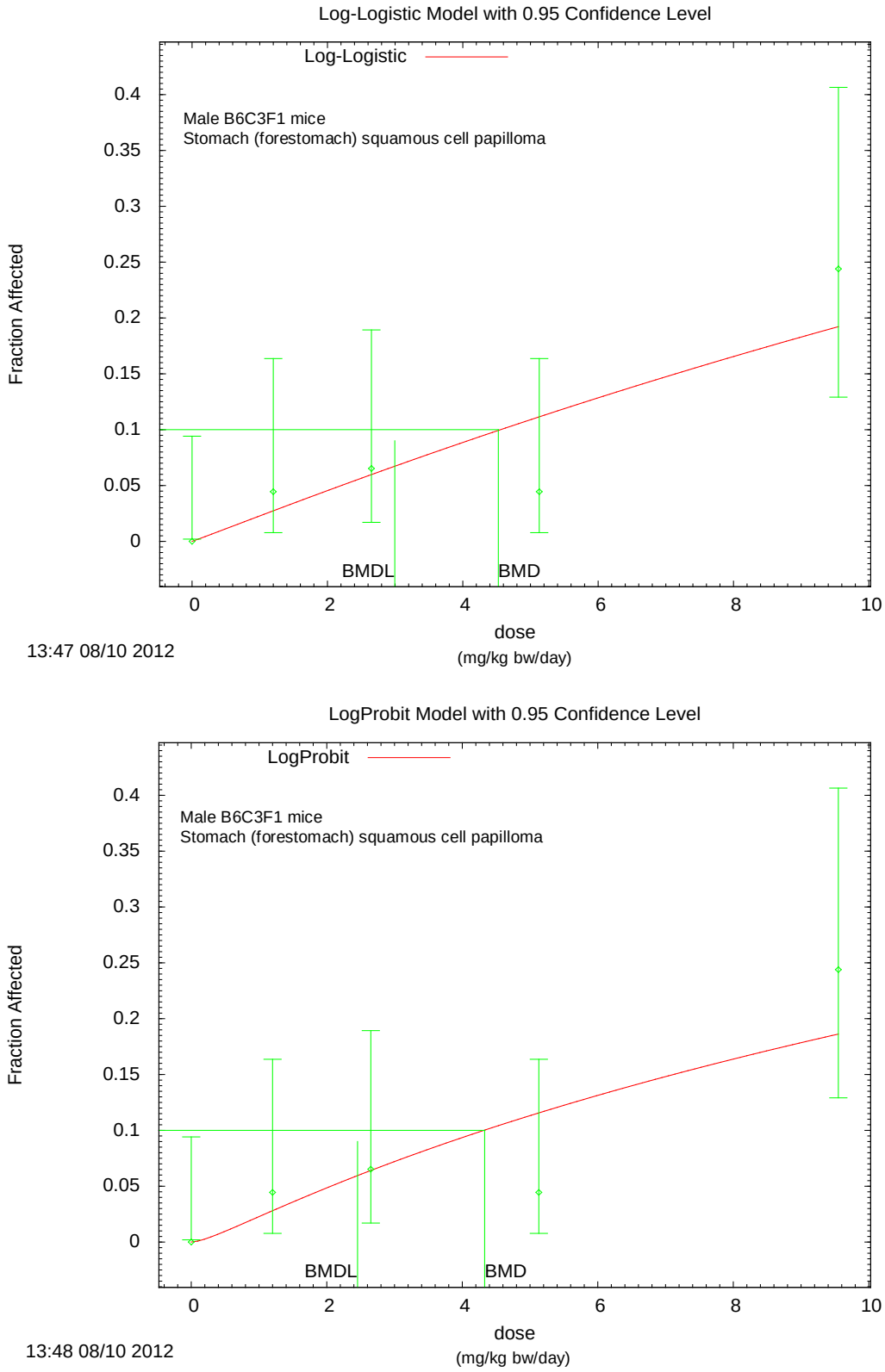


Figure K-34. Benchmark Dose Modeling of Stomach (Forestomach) Squamous Cell Papilloma in Male B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: Log-Logistic Model; Bottom: LogProbit Model)

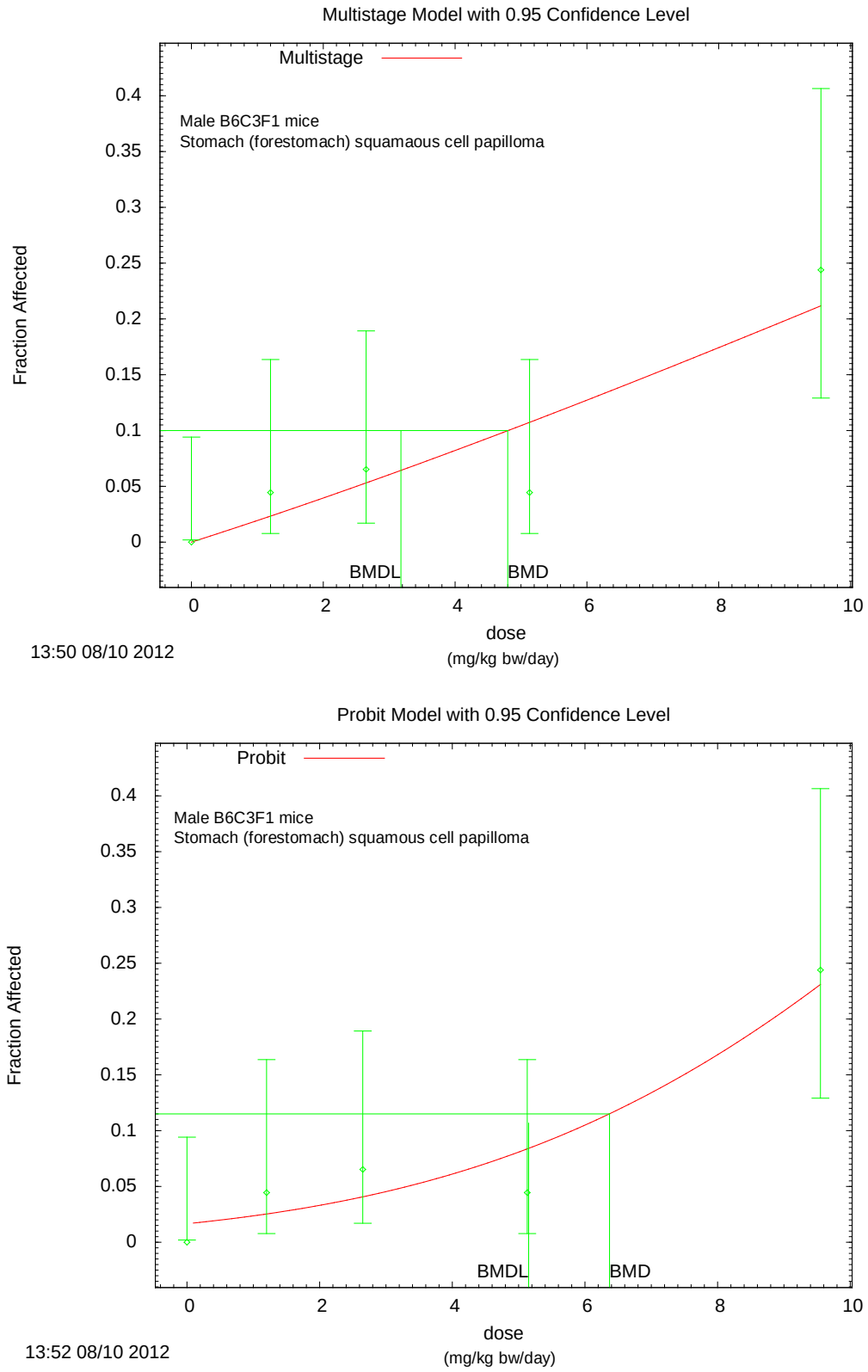


Figure K-35. Benchmark Dose Modeling of Stomach (Forestomach) Squamous Cell Papilloma in Male B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: Multistage Model; Bottom: Probit Model)

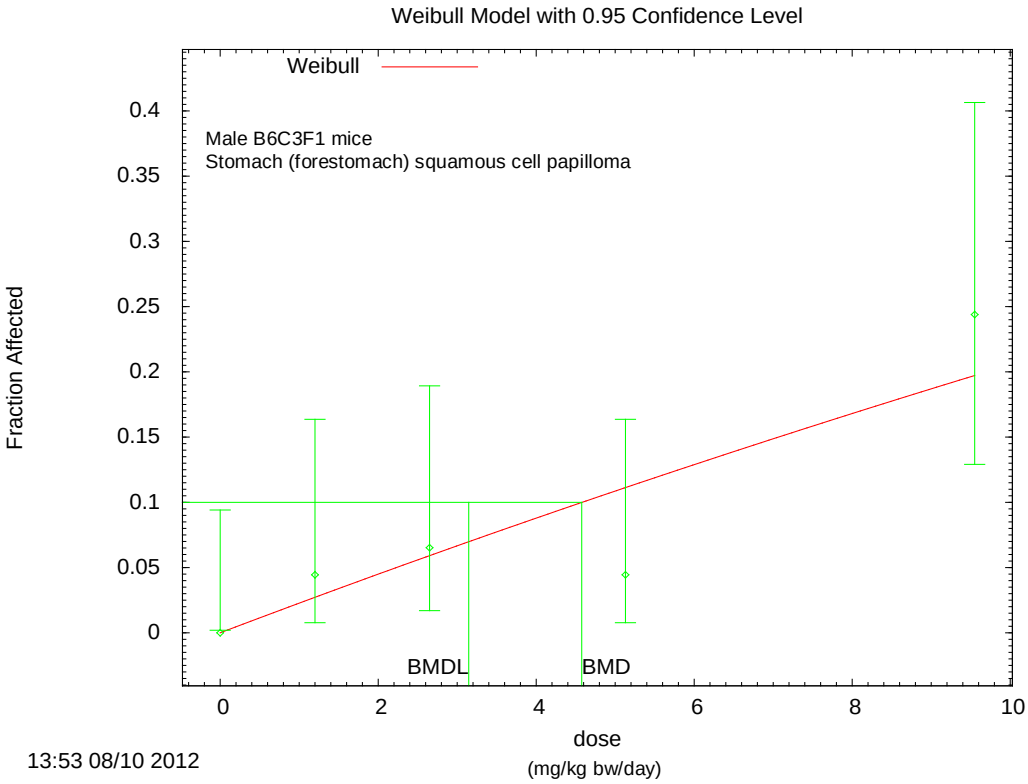


Figure K-36. Benchmark Dose Modeling of Stomach (Forestomach) Squamous Cell Papilloma in Male B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Weibull Model)

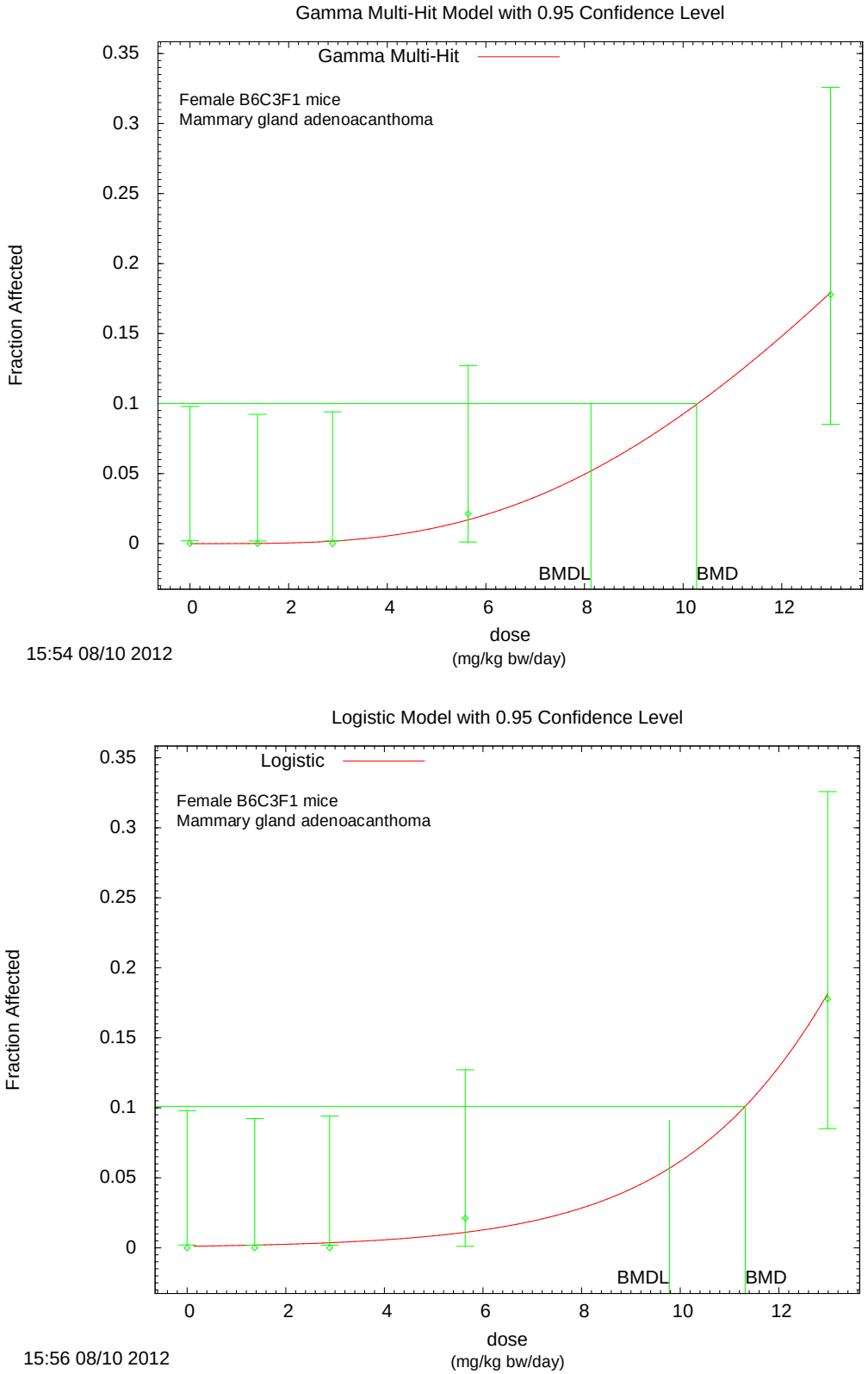
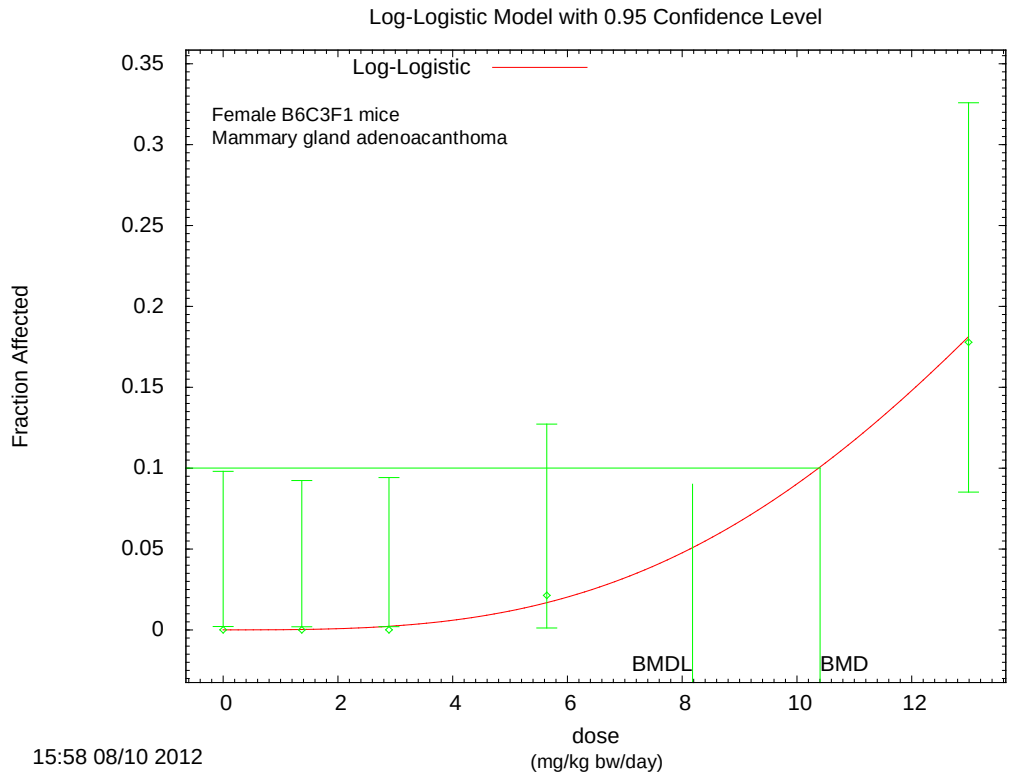


Figure K-37. Benchmark Dose Modeling of Mammary Gland Adenoacanthoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: Gamma Multi-hit Model; Bottom: Logistic Model)



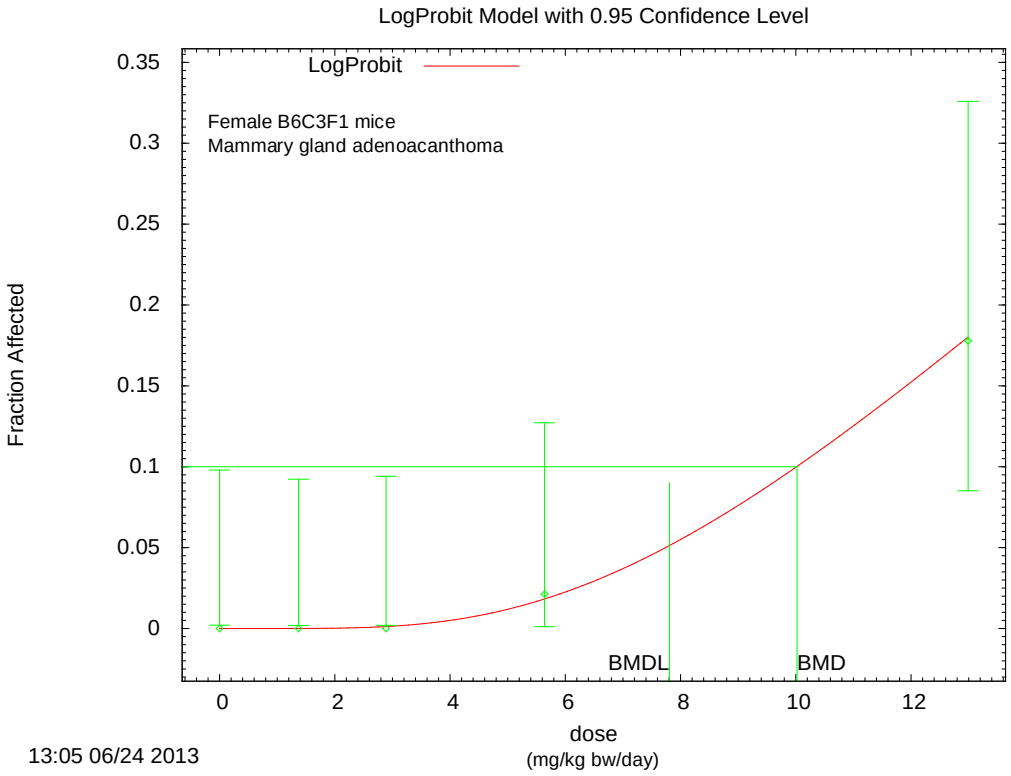
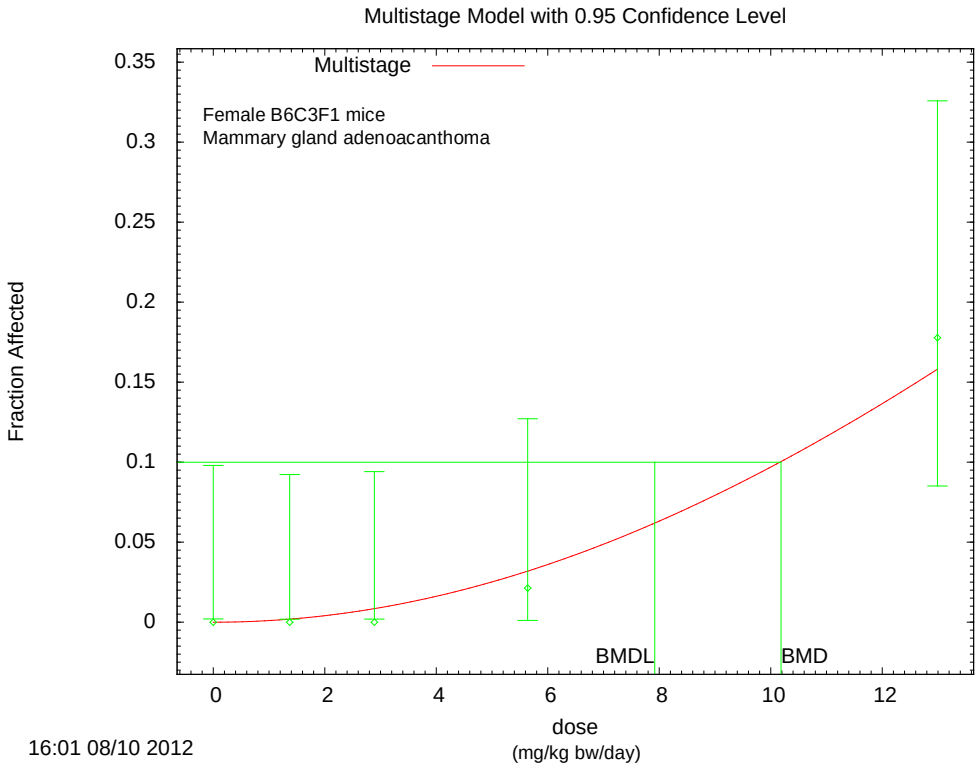


Figure K-38. Benchmark Dose Modeling of Mammary Gland Adenoacanthoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: Log-Logistic Model; Bottom: LogProbit Model)



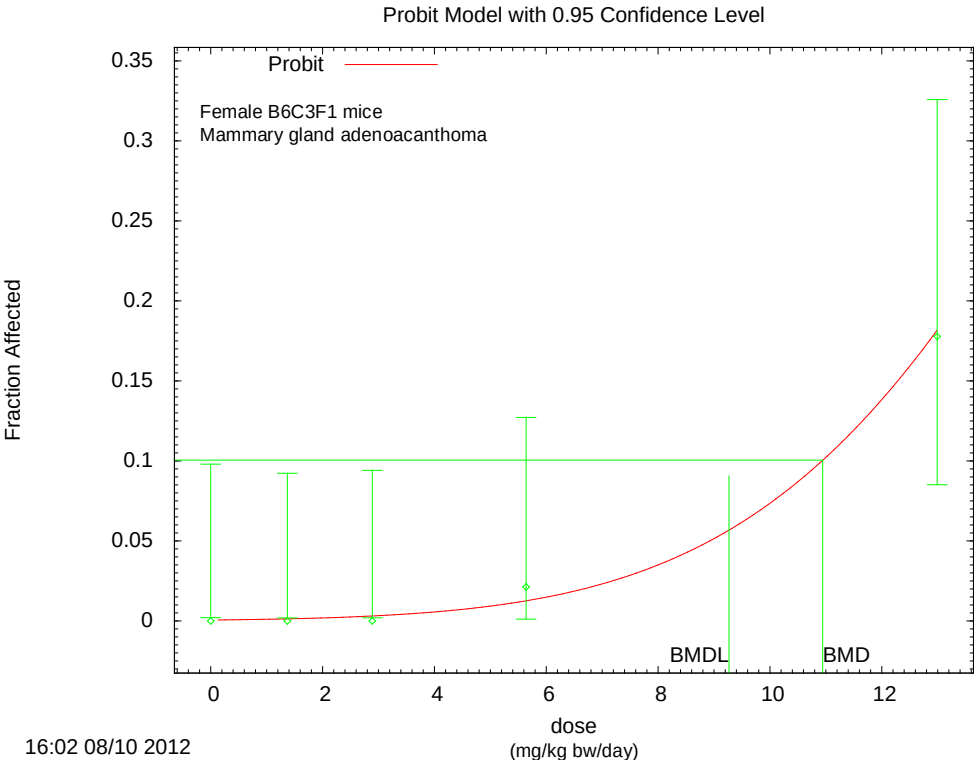


Figure K-39. Benchmark Dose Modeling of Mammary Gland Adenoacanthoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: Multistage Model; Bottom: Probit Model)

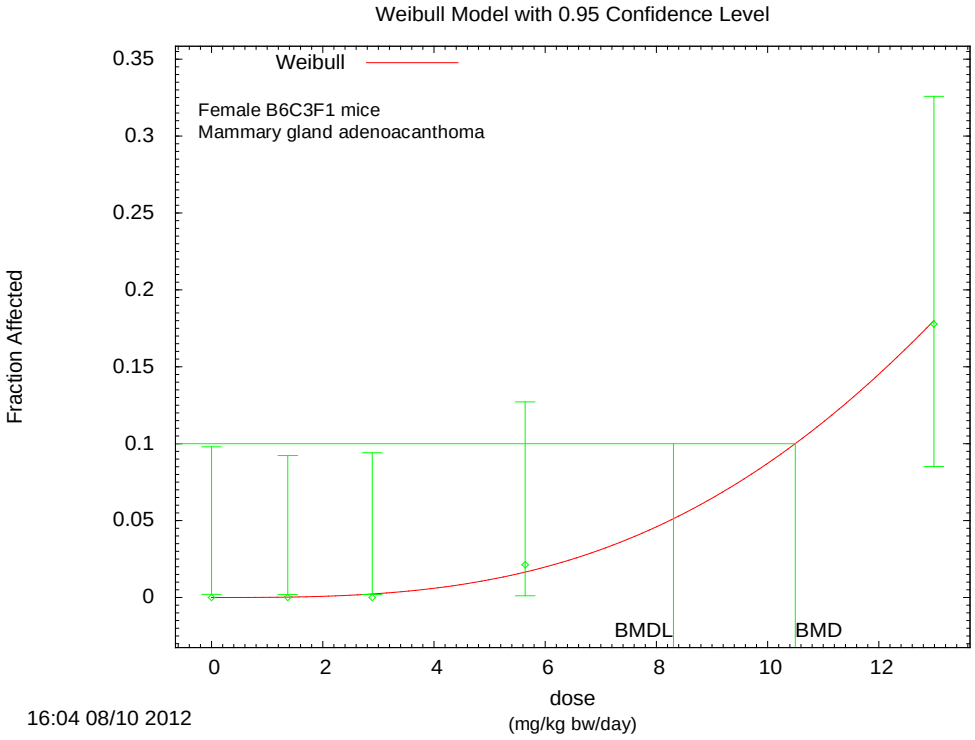


Figure K-40. Benchmark Dose Modeling of Mammary Gland Adenoacanthoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Weibull Model)

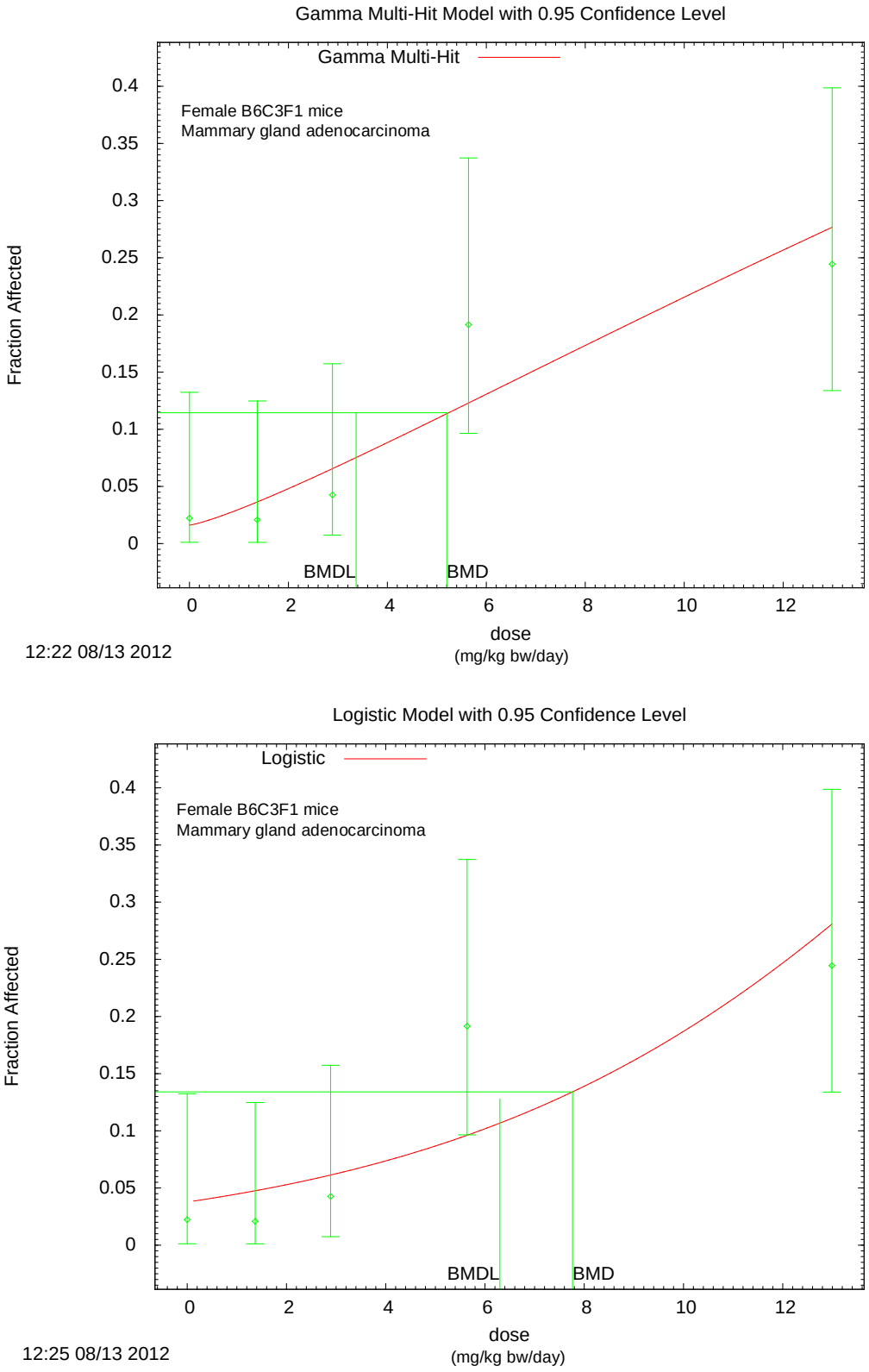


Figure K-41. Benchmark Dose Modeling of Mammary Gland Adenocarcinoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: Gamma Multi-hit Model; Bottom: Logistic Model)

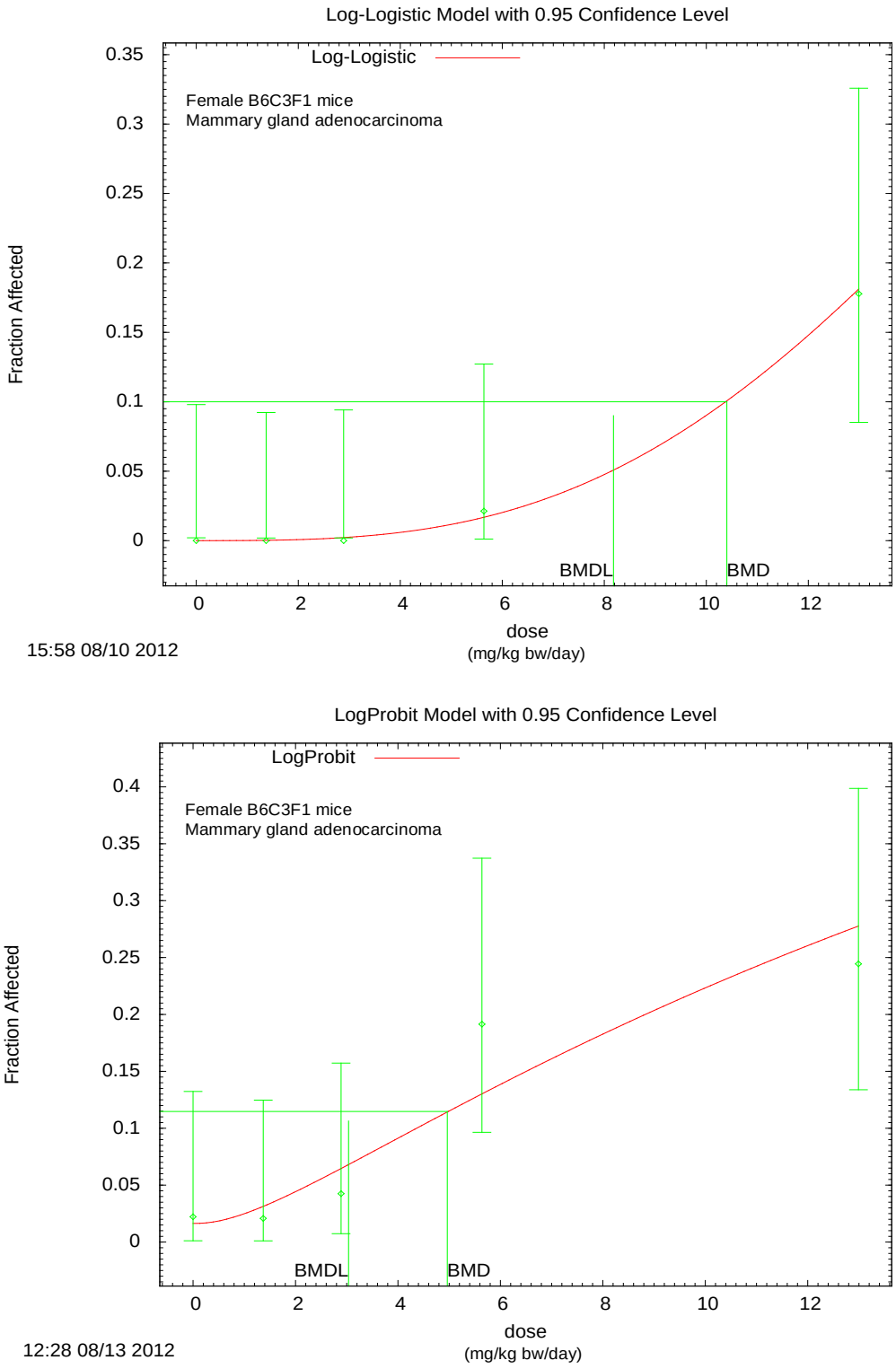
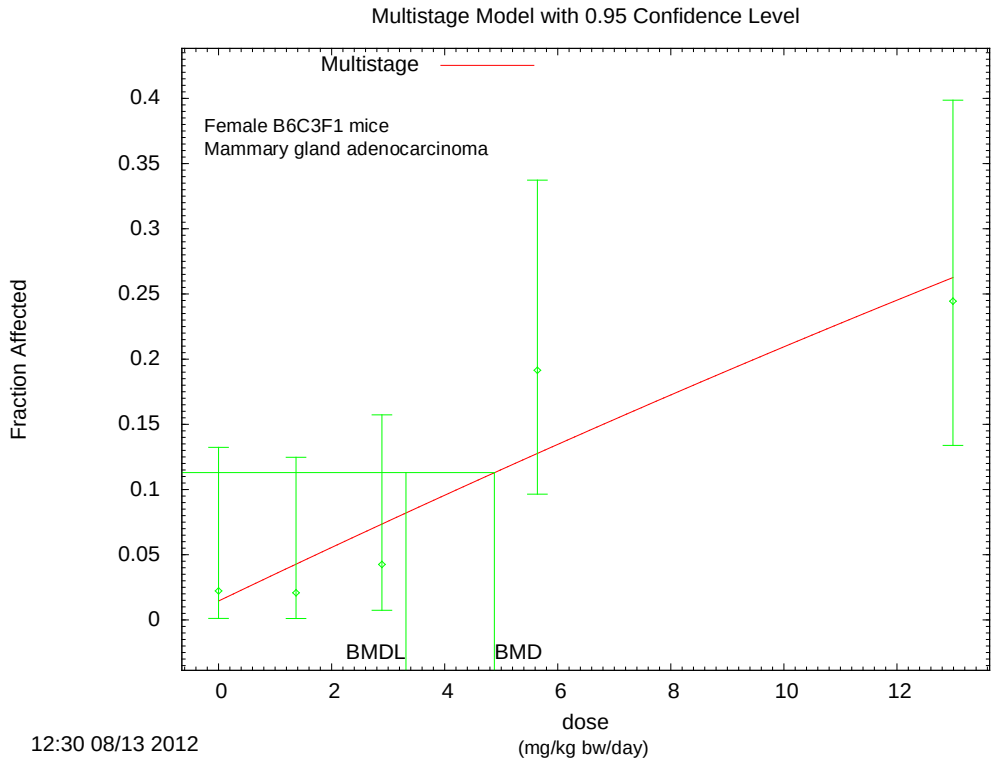


Figure K-42. Benchmark Dose Modeling of Mammary Gland Adenocarcinoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: Log-Logistic Model; Bottom: LogProbit Model)



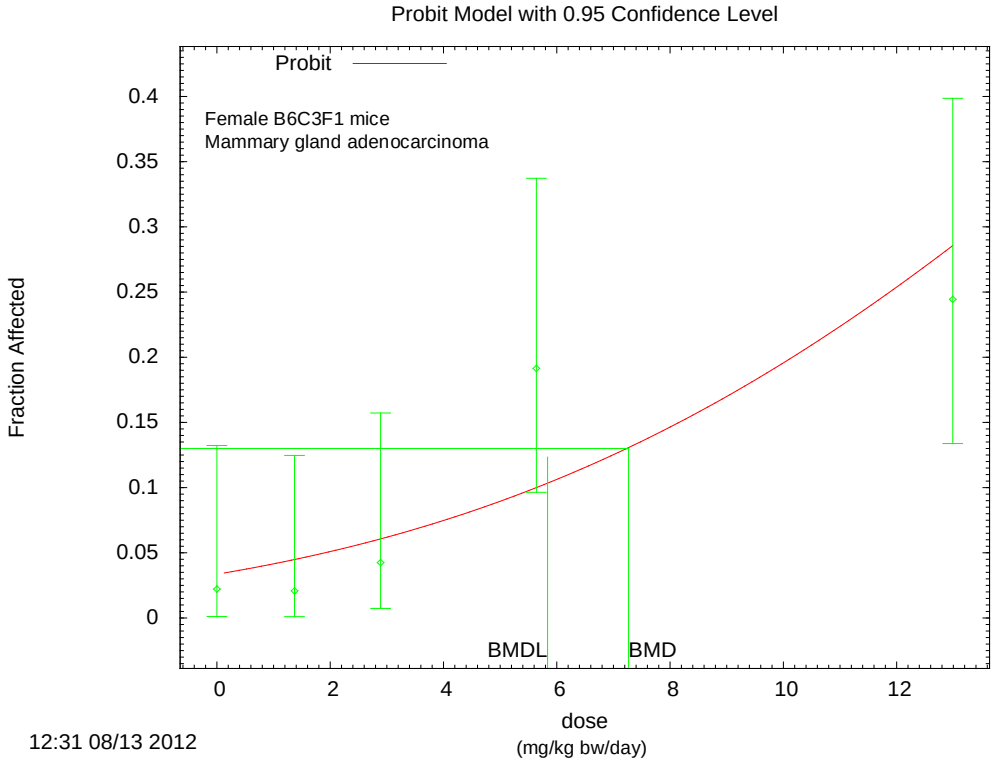


Figure K-43. Benchmark Dose Modeling of Mammary Gland Adenocarcinoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: Multistage Model; Bottom: Probit Model)

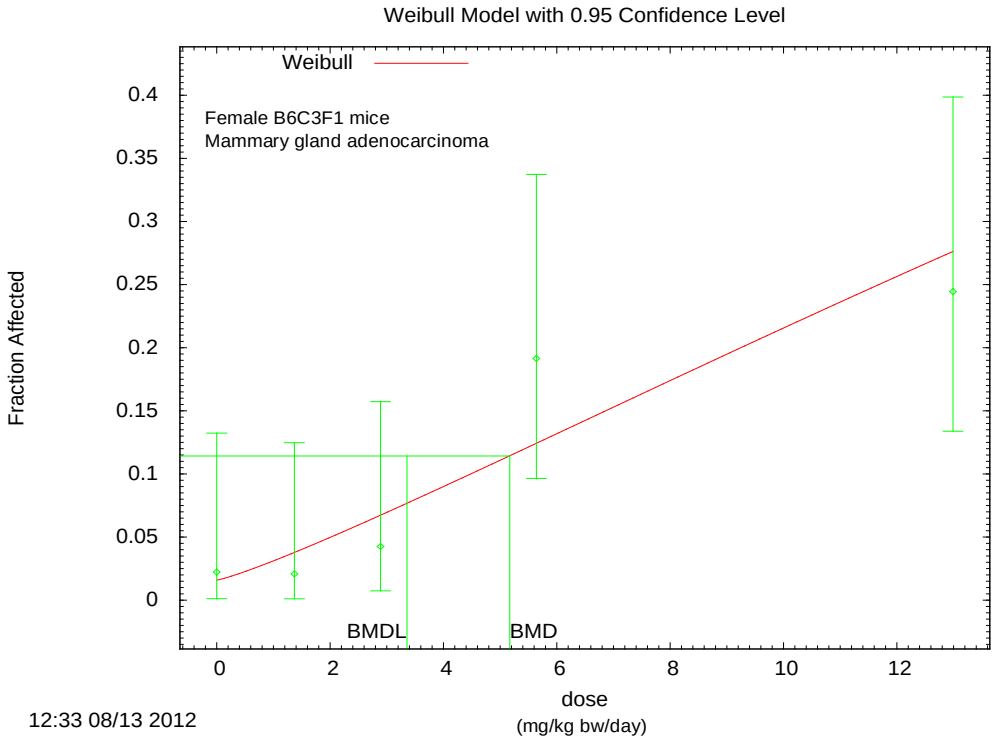


Figure K-44. Benchmark Dose Modeling of Mammary Gland Adenocarcinoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Weibull Model)

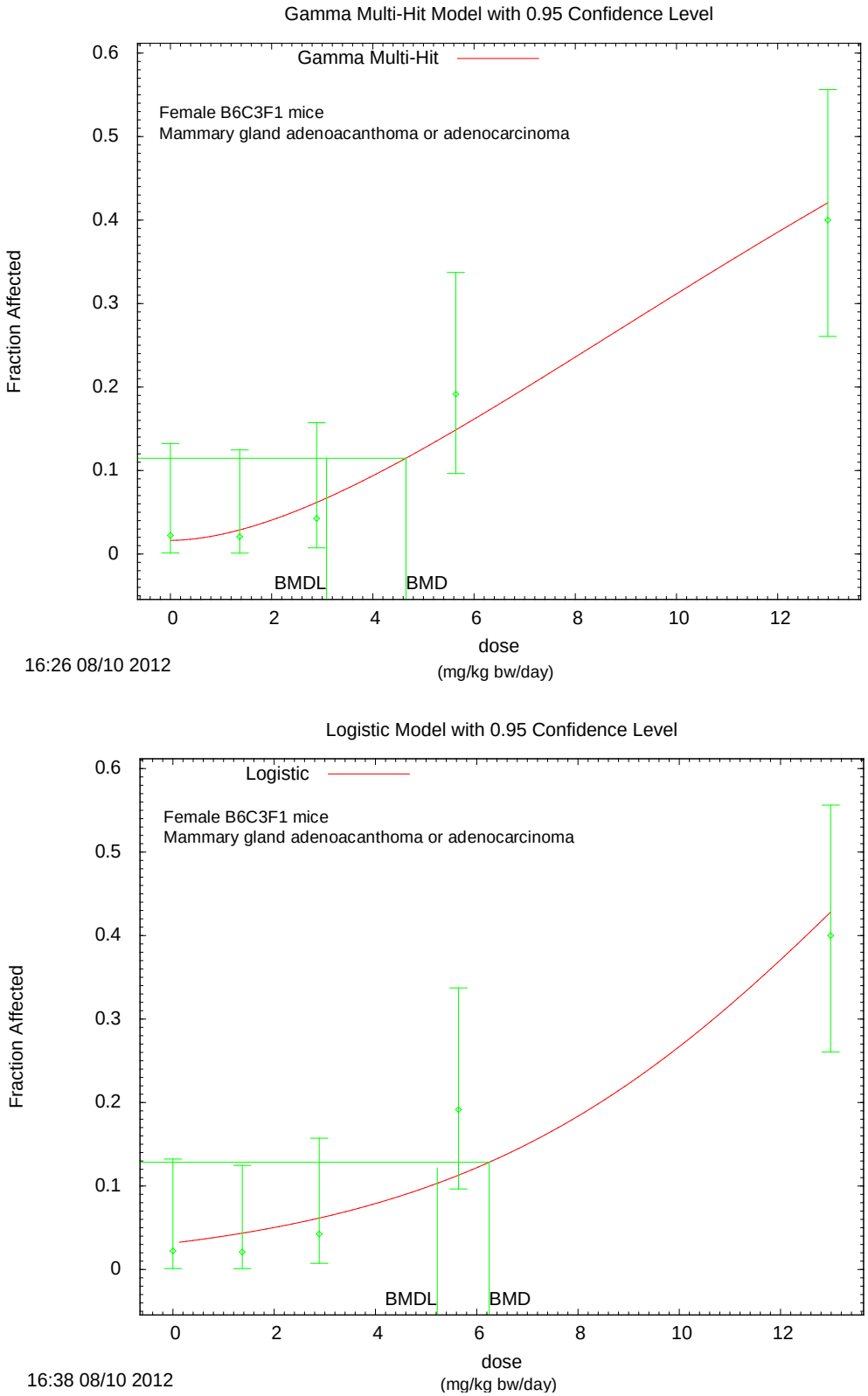
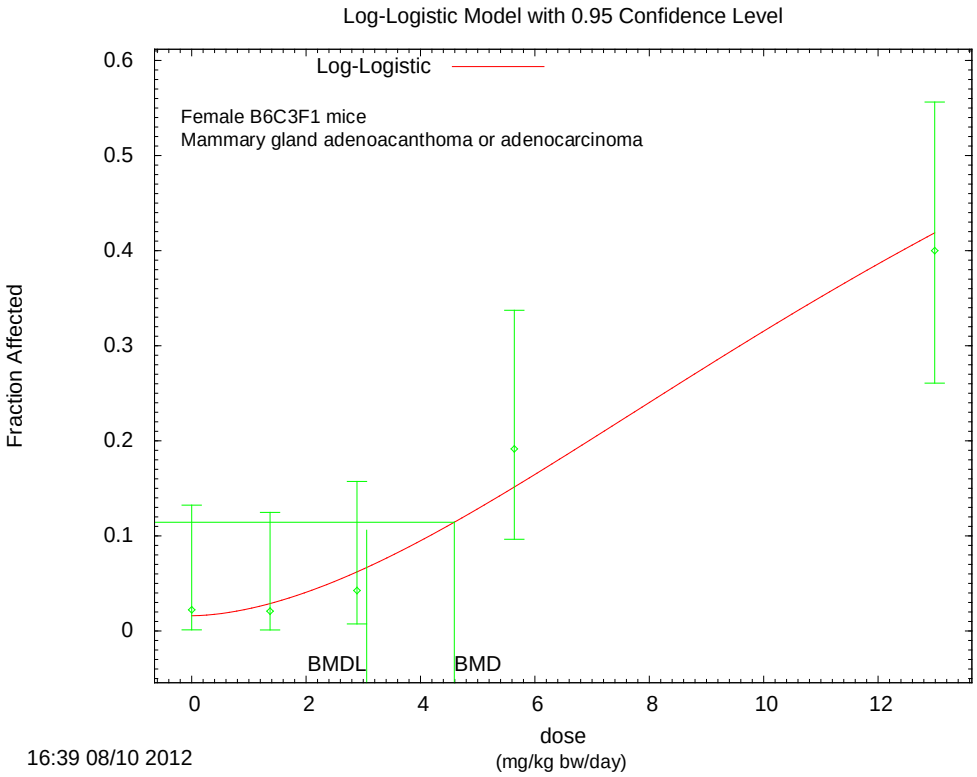


Figure K-45. Benchmark Dose Modeling of Mammary Gland Adenoacanthoma or Adenocarcinoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: Gamma Multi-hit Model; Bottom: Logistic Model)



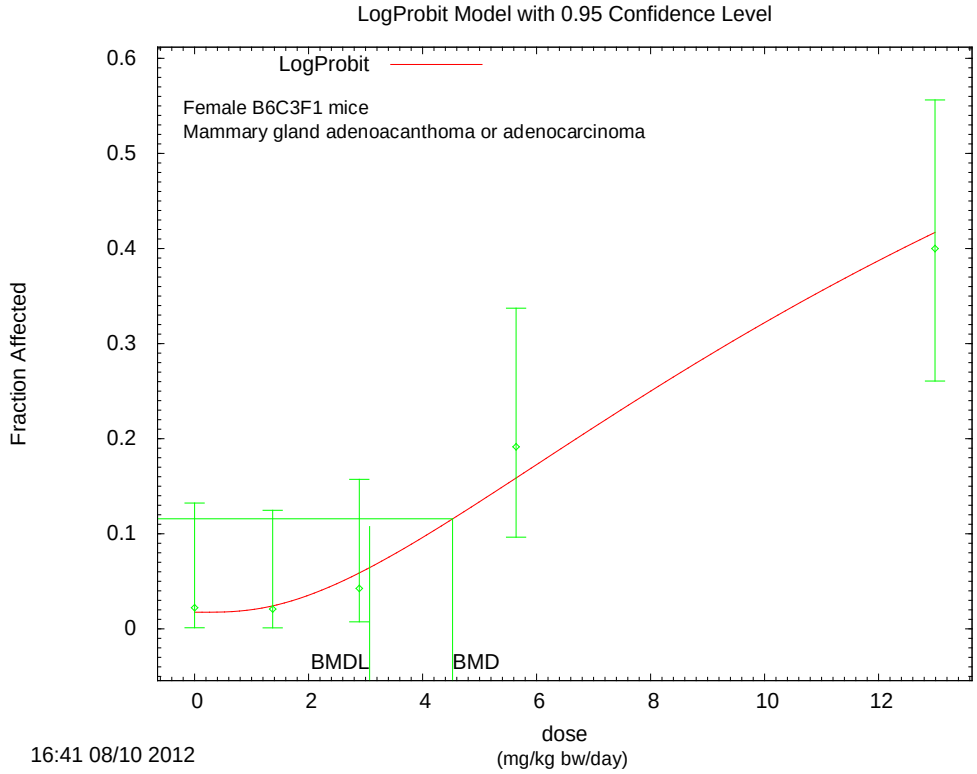
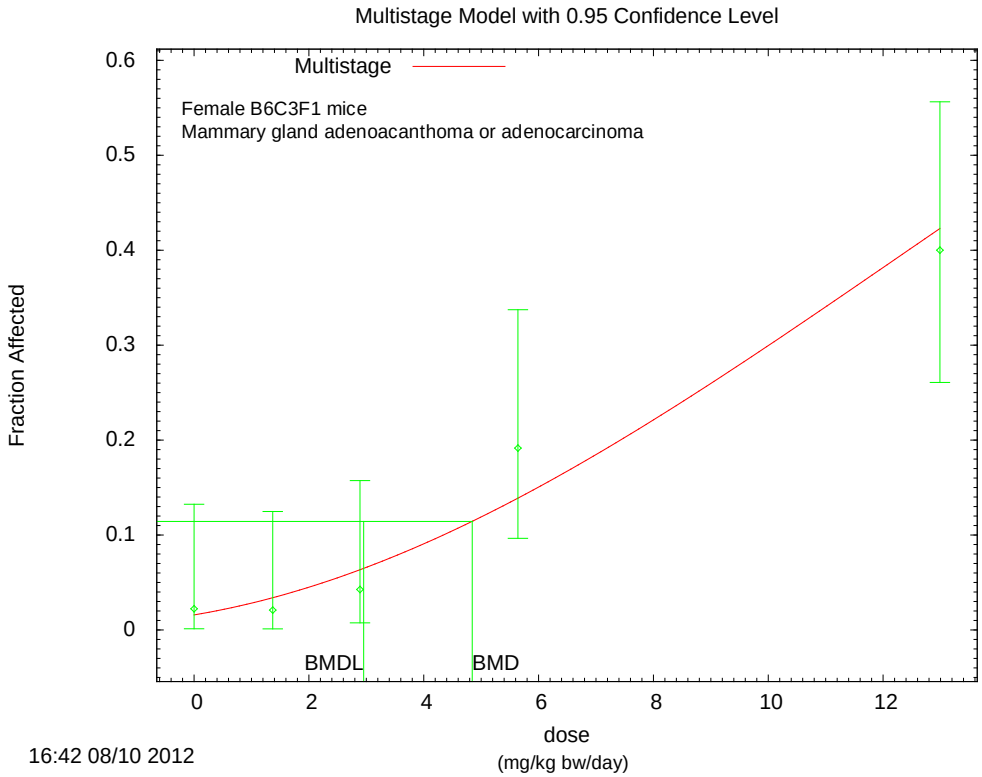


Figure K-46. Benchmark Dose Modeling of Mammary Gland Adenoacanthoma or Adenocarcinoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: Log-Logistic Model; Bottom: LogProbit Model)



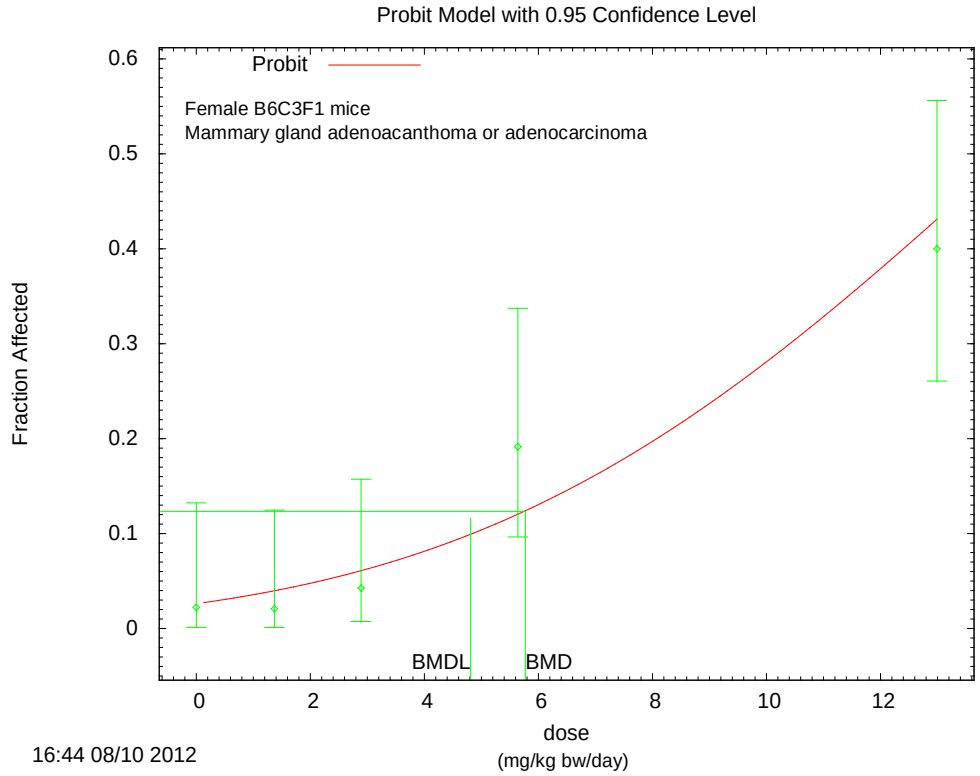


Figure K-47. Benchmark Dose Modeling of Mammary Gland Adenoacanthoma or Adenocarcinoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Top: Multistage Model; Bottom: Probit Model)

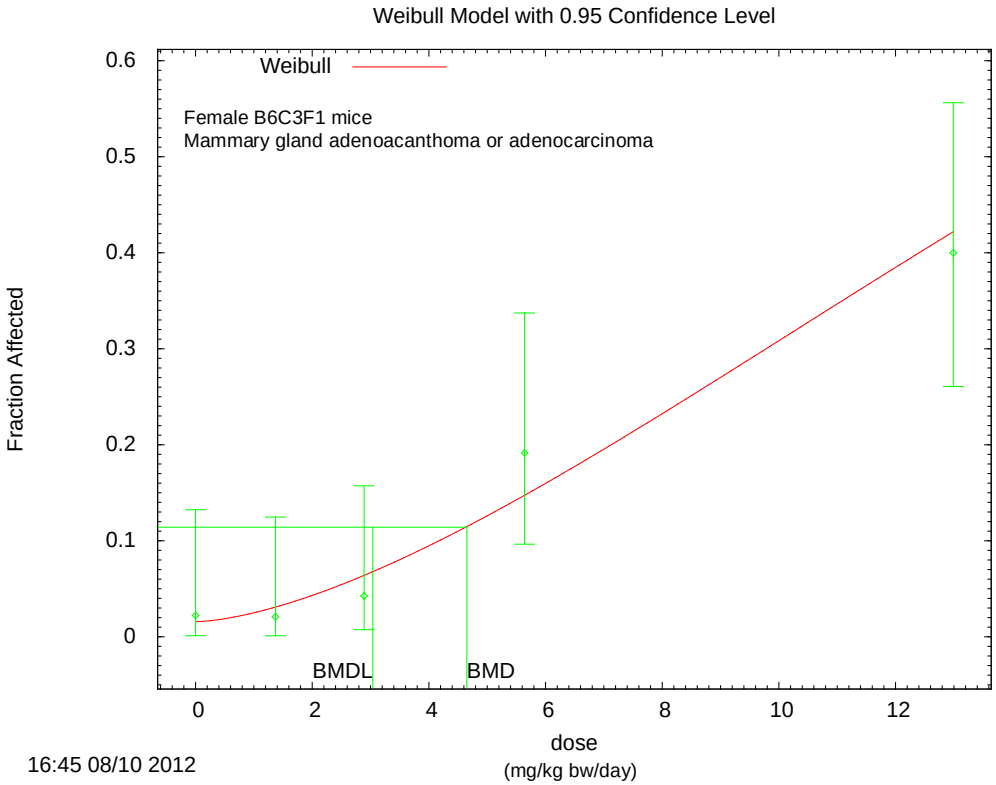


Figure K-48. Benchmark Dose Modeling of Mammary Gland Adenoacanthoma or Adenocarcinoma in Female B6C3F1/Nctr Mice in the Two-year Drinking Water Study of Glycidamide (Weibull Model)

Appendix L. Summary of Peer Review Panel Comments

On October 29, 2013, the draft Technical Report on the toxicology and carcinogenesis studies of glycidamide received public review by the National Toxicology Program's Technical Report Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. F.A. Beland, NCTR, introduced the toxicology and carcinogenesis drinking water studies of glycidamide by describing glycidamide as a metabolite of acrylamide, a contaminant present in certain baked goods and fried, starchy foods, as well as cigarette smoke. NTP performed parallel studies to determine and compare the long-term effects of acrylamide and glycidamide in rats and mice. Dr. Beland also described the design and result of the 2-week, 3-month, and 2-year studies. The proposed conclusions were *clear evidence of carcinogenic activity* in male and female F344/N Nctr rats and male and female B6C3F1/Nctr mice.

Dr. Cory-Slechta, the first primary reviewer, remarked that the studies were well done and that she agreed with the proposed conclusions.

Dr. Cattley, the second primary reviewer, noted that the presentation had clarified the dose selection rationale for glycidamide, but that the issue should be clarified in the Technical Report. He suggested adding historical control data for the incidence of alveolar/bronchiolar carcinomas in female mice to Table 13 and Appendix Table D-3. He noted that "gliosis" should be moved from neoplastic to nonneoplastic lesions in the Abstract Summary Table. He agreed in principle with the proposed conclusions; however, he suggested limiting "clear evidence" in rats to oral cavity squamous papillomas, because the incidence of oral cavity squamous papilloma or carcinoma (combined) is almost entirely derived from the papilloma and not the carcinoma incidence. Similarly, for mice, he suggested limiting the conclusion of "clear evidence" to alveolar/bronchiolar adenomas, because the combined incidence of alveolar/bronchiolar neoplasms is almost entirely derived from the adenoma and not the carcinoma incidence.

Dr. Barlow, the third primary reviewer, agreed that Dr. Beland's presentation had cleared up the issue of the dose rationale, and suggested the explanation should be added to the Technical Report. He noted that the Technical Report states there was decreased survival compared to controls due to tumors, but some of the tumors listed were not actually treatment-related and suggested clarification in the report. Dr. Barlow noted that only two males and females in the high-dose group survived to study termination, and asked whether the study should have been terminated earlier. He thought that oral cavity papillomas or carcinomas merited only "some evidence of carcinogenicity," rather than "clear evidence." He suggested deleting squamous cell papillomas as increased in the results text for female rats and changing the conclusion regarding squamous cell papillomas to "may have been related." He stated that axonal degeneration should not be as significantly highlighted as it was in the draft Technical Report. He asked whether the nonneoplastic findings listed in the conclusions are truly increased related to treatment. He had similar comments for mesenteric lymph node cellular infiltrate and pituitary gland hyperplasia. He also questioned the conclusion regarding Zymbal's gland carcinoma, because only a few animals were examined. He suggested that the increase in the incidences of alveolar/bronchiolar neoplasms in female mice should be listed as "considered related to," given a lack of clear dose response and only a mild increase at the high dose and that the benign granulosa cell tumors

should be combined with several malignant granulosa cell tumors to strengthen the statement, perhaps to the level of “some evidence.”

Dr. Cory-Slechta noted that Dr. Barlow seemed to discount the axonal degeneration because it appears in different places in the two sexes. She said that axonal degeneration is probably one of the best-documented effects of acrylamide in the neurotoxicology literature. She questioned why Dr. Barlow was discounting it. Dr. Barlow replied that he based his position on the doses that were used and the lack of a robust dose response.

Dr. Beland responded to Dr. Cattley’s comments. He would address the dose selection more thoroughly, add references to lung carcinomas, and correct the erroneous reference to gliosis in the Abstract Summary Table. Regarding the oral cavity tumors, he said squamous cell carcinoma is very rare in the control animals, so it was important to mention. Regarding the lung neoplasms in the mice, he said the conclusion states they were primarily adenomas, and he agreed to modify the text to describe that more clearly.

Regarding Dr. Barlow’s comment, Dr. Beland agreed to explain better that the two studies were conducted simultaneously. Addressing the comment about survival, he clarified that the animals were not removed due to overt toxicity or weight loss, noting that the veterinary staff monitored the animals very closely. Animals were removed because of spontaneous or treatment-related tumors. Given the need for direct comparison with acrylamide-treated animals, he proposed it was permissible to keep animals on the study until tumor development dictated their removal.

Regarding the suggestion that the call be changed for oral cavity tumors in rats, Dr. Beland stated that the response was robust and monotonic. He believed that the conclusions regarding the clitoral gland and forestomach tumors were correct. He noted that there was much interest in neurotoxicity of glycidamide and acrylamide, and the axonal degeneration was included to demonstrate that a careful examination for potential neurotoxicity in the animals was conducted. He said that the squamous cell papillomas discussed in the Results text for female rats would be deleted.

Regarding Zymbal’s gland tumors, Dr. Beland said if a lesion were observed during necropsy, the histopathology would be conducted. The statistics were compiled according to how many animals have such a tumor versus the entire cohort.

Regarding the suggestion to change the call on the lung neoplasms in female mice, Dr. Beland noted that the two highest doses exceeded the historical control by two- to three-fold, so “clear evidence” was proposed. Statistical analysis combining the benign granulose cell tumor with other malignant granulosa cell tumors indicated no significant effect.

Dr. Cullen asked how Dr. Beland would explain the lack of esophageal problems, given the mechanism of action. He also asked whether it was correct to assume that fibroadenomas do not have a high risk of converting into mammary carcinomas. Dr. Cullen understood the requirement for “clear evidence” of benign tumors is that it be a benign tumor with a high risk of conversion into a malignant form. He felt the call of “clear evidence” on the fibroadenomas might be overreaching.

Referring to the lack of esophageal problems, Dr. Beland said he could not explain why cancer did not occur. He recalled discussion in the acrylamide study about fibroadenomas and the

possibility of progressing to a malignant tumor. Dr. Bucher, NIEHS, commented that the definition of clear evidence includes the presence of a marked increase in benign tumors. Dr. Beland was hesitant to change the clear evidence call because several regulatory agencies are using fibroadenomas in developing risk estimates for acrylamide. Editorial changes will also be made to the conclusions.

Dr. Malarkey, NIEHS, noted that axonal degeneration is a common background lesion in mice and rats, so what is being sought is exacerbation beyond background levels, which did not occur in this study.

Dr. Cullen called for a motion to accept the conclusions in the draft report as written. Dr. Cory-Slechta moved to accept the conclusions as written and Dr. Gordon seconded. The peer review panel voted (six in favor, one opposed, and zero abstentions) to accept the conclusions on glycidamide as written in the draft report. Dr. Cattley explained that his negative vote was based on the combination of papilloma and carcinoma for oral cavity lesions in the rats, and the combination of adenomas and carcinomas in alveolar/bronchiolar lung neoplasms. He proposed that both of those responses for “clear evidence” are based on the benign neoplasm, not the malignant neoplasm.

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