UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

AUG 28 2002

MEMORANDUM

SUBJECT: Zeomic® Type AJ10D Silver Zeolite A: Review of Toxicology data submitted.

EPA Identification Numbers:

P.C. Code:072503
DP Barcodes:D281584

MRID's: 45581105, -06, -07
Submissions: S612009

TO: Dennis Edwards / Marshall Swindell / Tony Kish
Regulatory Management Branch I / PM Team 33
Antimicrobials Division (7510C)

FROM: Timothy F. McMahon, Ph.D. 8/28/02
Senior Toxicologist
Antimicrobials Division (7510C)

THRU: Nader Elkassabany, Ph.D. Nader Elkassabany 8/28/02
Acting Team Leader, Team Two
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510C)

and

Norm Cook, Chief 08.28.02
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510C)

Action Requested: Review of the submitted toxicology data matrix for Zeomic® Type AJ10D Silver Zeolite A.

Background

Two subchronic toxicity studies and one chronic toxicity/carcinogenicity study for Zeomic® Type AJ10D Silver Zeolite A were submitted by the registrant (Sinanen Company, Ltd.) The executive summaries of these studies are presented below.

CITATION: Serota, D.G. (2001) 90-Day Dietary Toxicity Study of Zeomic in Rats. MPI Research, Inc. (Mattawan, Michigan). Study No. 892-001, December 20, 2001. MRID 455811-05. Unpublished.

EXECUTIVE SUMMARY

In a subchronic toxicity study (MRID 455811-05), Zeomic (unspecified purity) was administered to CD® [Crl:CD® (SD) IGS BR] rats (10/sex/group) in the diet at 0, 1000, 6250, and 12,500 ppm for 13 weeks. This reviewer calculated the doses to be the following: (males), 0, 62, 392, and 840 mg/kg-day; (females) 0, 74, 487, and 933 mg/kg-day based on body weight and food consumption data provided in the study report. The oral route of exposure was chosen because it is a possible route of human exposure. Doses were chosen based on data from previous studies involving this test article. Details regarding those studies were not reported.

No treatment-related effects on mortality, clinical observations, neurobehavioral parameters, Functional Observational Battery (FOB) evaluations, motor activity measurements, food consumption, ophthalmology, or organ weights were observed. Treatment-related findings include the following. At 12,500 ppm (840 mg/kg-day), males gained approximately 10% less body weight over the study duration compared with controls, and animals of both sexes displayed dose-related changes in erythrocytic parameters that may have been related to zinc toxicity (the changes were more pronounced in males). Decreased urine volume in both males and females, and increased urinary pH in males were observed at 12,500 ppm. At both 6250 and 12,500 ppm, a dose-dependent, treatment-related increase in alkaline phosphatase and elevated cholesterol levels were observed in males and females (cholesterol levels in males also were elevated at 1000 ppm). The study authors questioned the biological/toxicological significance of the increased cholesterol because the increases were not correlated with body weight or food consumption changes at 1000 or 6250 ppm in males or at 6250 or 12,500 ppm in females. Also at both 6250 and 12,500 ppm in both males and females, a green discoloration of the pancreas was noted that was correlated with pigmentation of the interstitial tissue. The incidence and severity of this finding was dose related, and the effect was more severe in females. However, because no cell injury was noted the study authors concluded that the pigment was inert. Based on the morphology of the pigment, the study authors concluded that it was exogenous and may have come from the silver component of Zeomic. Even though a statistically significant and dose-related increase in cholesterol was observed in males at 1000 ppm, this observation was not corroborated by any other parameters measured, such as mortality, macroscopic, or microscopic pathology. By contrast, the hematological alterations seen at the 6250 ppm dose level (decreased hemoglobin, hematocrit, MCV, MCHC) are suggestive of possible zinc toxicity. Zinc can interfere with absorption of copper, and low copper intake has been linked to anemia. **Thus, based on the results of this study, the LOAEL is determined to be 6250 ppm (392 mg/kg/day [M]; 487 mg/kg/day [F], and the NOAEL is determined to be 1000 ppm (62 mg/kg/day [M]; 74 mg/kg/day [F].**

This subchronic toxicity study is classified as **acceptable/guideline**, with deficiencies in the study being discussed in the "Study Deficiencies" section of this evaluation report.

CITATION: Hirasawa, F., Takizawa, Y., Tsunoda, H., Uesugi, S., Fjuii, M. (1994) Studies on silver, zinc and ammonia complex substitution type A zeolite from the perspective of public health studies I: subchronic toxicity test. Japanese Journal of Food Chemistry, Vol.1(1). MRID No. 455811-06.

EXECUTIVE SUMMARY

In a subchronic toxicity study (MRID 45581106), silver, zinc and ammonia complex substitution type A zeolite (AZN zeolite) (purity not provided) was administered to 100 four-week old Fischer-344 rats (ten/sex/group) in the diet for 13 weeks. Rats were divided into five groups: control, AZN zeolite 1.25% feed mixture, AZN zeolite 2.5% feed mixture, AZN zeolite 5.0% feed mixture, and satellite (group to which a four-week recovery period was provided following administration of AZN zeolite 5.0% feed mixture).

Clinical signs of toxicity included poor skin complexion, reduced spontaneous movement, emaciation, and tremors, beginning during weeks 7-8. Two premature deaths occurred during weeks 11 and 13 of the 13-week administration period, and six premature deaths occurred during weeks 14 and 15 of the four-week recovery period. Five of the six premature deaths that occurred during the recovery period resulted from atrial thrombosis, which the study authors attributed to the toxicity of the test material. All premature deaths involved animals that were administered 5.0% AZN zeolite feed mixture. Treatment-related effects were observed in body weight gain, food consumption, hematology, clinical chemistry, and histopathology. Following discontinuation of administration of AZN zeolite, body weight gain and food consumption improved. Significant dose-dependent patterns were observed for many hematological and clinical chemistry parameters. Histopathology revealed pigmentation of numerous organs and fibrosis and atrophy of the pancreas. **The study authors did not determine NOAELs or LOAELs. Our reviewers concluded that the LOEL is 1.25% based on: significantly lower values for body weight gain and food consumption, dose-dependent patterns for many hematological and clinical chemistry components, pigmentation of numerous organs, and other non-tumorigenic changes. Our reviewers were unable to convert this value to mg/kg-day because actual doses were not provided and concentrations in the diet could not be converted to doses.**

This subchronic toxicity study is classified as **unacceptable** because evaluation of certain parameters (e.g., functional observations, ophthalmology, organ weights) necessary for the adequate assessment of subchronic toxicity was not conducted, and evaluation of other parameters (e.g., histopathology) was incomplete. Furthermore, no stability, homogeneity, or concentration analyses were reported for the test diets, which prevents an adequate assessment of the amount of test material that the animals received during the course of the study and therefore assignment of NOAEL/LOAEL values. Therefore, this study does not satisfy the guideline requirements for a subchronic oral study (OPPTS 870.3100) in rodents. The study is **not upgradeable** as discussed in the study deficiencies section of this evaluation report.

CITATION: **First report**: Takizawa, Y. (1994). Combined Chronic Toxicity/Carcinogenicity Study of Zeomic in Mice and Rats. Depatment of Public Hygiene, Akita University, School of Medicine. (Japan). April 25, 1994. MRID 45581107. Unpublished.

Second report: Takizawa, Y., Hirasawa, F., Fujii, M., et al. (1995). A Study on Oral Chronic Toxicity and Carcinogenicity of Silver-, Zinc-, Ammonia-Complex-Substituted Zeolite. (Japan). Accepted on March 20, 1995. Unpublished.

EXECUTIVE SUMMARY

Two studies (MRID 4551107) are reviewed in this DER, both using Zeomic, containing 2.3% Ag, 12.5% Zn and 2.5% NH4.

In the first study, a combined chronic toxicity/carcinogenicity study, B6C3F1 (SPF) mice (50 sex/dose plus additional animals in satellite groups for interim examination) were fed Zeomic at dose levels of 0, 0.1%, 0.3% or 0.9% for 24 months. These doses were calculated by this reviewer to correspond to 106, 340 and 1015 mg/kg bw/day for males based on data provided on nominal concentrations, food intake and body weights. A significant portion of the female data were missing and similar calculations for dose conversions were not possible. In the chronic portion of this study, the most consistent and sensitive effects were observed on hematological parameters at the 0.3% dietary level (decreased RBC, HBG, HCT and MCV in both sexes, although females appeared slightly more sensitive). The NOAEL for these effects was 0.1% for females (calculated by this reviewer to be 106 mg/kg bw/day for males) and the LOAEL was 0.3% (calculated by this reviewer to be 340 mg/kg bw/day for males). There were also effects on organ weights, i.e., increased pancreatic weights in males at 24 months, the NOEL being 0.1% (106 mg/kg bw/day) and the LOAEL = 0.3% (340 mg/kg bw/day) and various non-neoplastic histopathological observations, e.g., enlargement of Langerhans' island in males and ovarian cysts in females. These histopathological effects are difficult to interpret in that all animals that died on study and those at terminal sacrifice were combined into one summary table (see relevant section of DER foradditional information). **The NOAEL for chronic toxicity in mice was determined to be 0.1% (106 mg/kg bw/day) and the LOAEL was 0.3% (340 mg/kg bw/day) based on the above changes in hematology and organ weight changes.**

The chronic toxicity study in mice is classified **unacceptable** and does not satisfy the guideline requirement for a chronic oral toxicity study in mice (OPPTS 870.4300). There were several deficiencies observed in this study, including no clinical chemistry determinations, no urinalyses, no opthalmological exams, missing or under-reporting of data (e.g., missing body weights for females for weeks 0-17 and 40-70), and some missing organs for histopathological exam. These data would be required in order to consider the study acceptable for regulatory purposes. Additional details may be found in the deficiency section of the DER as well as in "Appendix A, Data Validation."

In the second portion of this study, the carcinogenic potential of Zeomic in mice was evaluated. Based on the data as presented, there appears to be no increase in tumors in this study. However, the tumor data (as well as the non-tumor data) are summarized by combining all animals that died on study (or sacrificed moribund) with those that were sacrificed at termination. Thus, without a separate analysis of these two

groups, a final assessment of the carcinogenic potential of Zeomic in this study is difficult. Although there was a lack of significant toxic effect at the high dose in mice, this dose appears to approximate a limit dose (1000 mg/kg). Thus, the data on carcinogenicity in mice may be useful in the future for determination of the carcinogenic potential of Zeomic.

This carcinogenicity study in mice is **unacceptable** in that it was not conducted under GLPs. Lack of assignment of a quality assurance unit is a significant deviation from GLP guidelines and is a basis for study rejection The study does not currently satisfy the guideline requirement for a carcinogenicity study in mice (OPPTS 870.4300). However, in that the males were exposed to the approximate Limit Dose, this study may provide useful information on the potential carcinogenicity of Zeomic in mice. If the tumor data are presented in proper format, e.g., with animals that died on study summarized by time of death separately from those that were sacrificed at termination, the study would be more useful in this respect. Also deficiencies in under-reporting listed in "Appendix A, Data Validation" should be addressed to the extent possible, e.g., clarification of exactly which tissues underwent a histopathological examination. Clear dose conversions from % to mg/kg bw/day should also be provided by the submitter. Pending receipt and review of this additional information, the final utility of these data in the evaluation of carcinogenicity remains uncertain.

In the second study, also a combined chronic toxicity/carcinogenicity study, Fisher 344 (SPF) rats (50 sex/dose in the main study group plus additional animals in satellite groups for interim examination) were fed Zeomic at dose levels of 0, 0.01%, 0.03%, 0.1% or 0.3% for 24 months. These doses were calculated by this reviewer, based on nominal concentrations in the diet, food intake and body weights to be 4.1, 12.2, 39.5 and 121 mg/kg bw/day for males and 4.1, 12.5, 41.1 and 125 mg/kg bw/day for females.

In the chronic portion of this study, consistent effects were again observed on hematological parameters (decreased HBG, HCT, MHC and MCHC in both males females at 24 months, although females again appeared more sensitive). There were other biochemical effects noted in this study (e.g., increased ALT and ALP), but they occurred at higher doses than those that affected the hematological parameters and there were no corresponding histopathological changes in the liver. **The NOAEL for chronic toxicity this study was 0.03% or 12.5 mg/kg bw/day (based on changes in hematological parameters in female rats) and the LOAEL = 0.1% or 41.1 mg/kg bw/day.**

The chronic portion of the rat study is **unacceptable** since it was not conducted under GLPs and other deficiencies were noted, such as no urinalysis, no ophthalmological exams, combining histopathology for non-neoplastic and neoplastic lesions from all animals that died on study with those sacrificed at termination, and significant portions of missing or under-reported data as outlined in "Appendix A, Data Validation".

In the second portion of this study, the carcinogenic potential of Zeomic in rats was evaluated. Based on the data as presented, there appears to be no increase in tumors in this study. There was an increase noted in leukemia (females) and endometrial polyps which were statistically significant for trend and/or by pair-wise comparison. These effects were dismissed in the report as being within the range of historical controls. These historical control data were not provided for review. In addition, the tumor data (as well as the non-tumor data) are summarized by combining all animals that died on study (or sacrificed moribund) with those that were sacrificed at termination. Thus, without a separate analysis of these two groups, a final assessment of the carcinogenic potential of Zeomic in this study is difficult.

Finally, there was not sufficient toxicity observed to consider that the high dose was adequate for carcinogenicity testing.

This carcinogenicity study in rats is **unacceptable** in that it was not conducted under GLPs. Lack of assignment of a quality assurance unit is a significant deviation from GLP guidelines and is a basis for study rejection. With regard to the carcinogenicity data, these data may be viewed as supplementary but the study does not satisfy the guideline requirement for a carcinogenicity study in rats (OPPTS 870.4300). There are no data to support that the top dose represented or even approximated an adequate dose for carcinogenicity testing. Survival was not adversely affected at the high dose nor were there any effects that could be supported as toxicologically significant.

DATA EVALUATION RECORD

SILVER, ZINC, AND AMMONIA COMPLEX SUBSTITUTION TYPE A ZEOLITE

Study Type: Subchronic Oral Toxicity (Rat)

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

ICF Consulting Group
9300 Lee Highway
Fairfax, VA 22031

Under Subcontract to

Versar
6850 Versar Center
P.O. Box 1549
Springfield, VA 22151

Principal Reviewer	Krystina S Hawryluk *Krystina S. Hawryluk, B.S.*	Date 6-3-02
Independent Reviewer	Jennifer Welham *Jennifer Welham, M.S.*	Date 6-3-02
ICF Program Manager	Kara D. Altshuler *Kara Altshuler, Ph.D.*	Date 6-4-02
Versar Program Manager	Linda Phillips *Linda Phillips, Ph.D.*	Date 6-7-02

Contract Number: 68-W-01-036
Work Assignment No.: 0248.2000.002.02
EPA Work Assignment Manager: Cletis Mixon, Ph.D.

EPA Reviewer: Tim McMahon, Ph.D. Date 2-29-12
Antimicrobials Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: Subchronic Oral Toxicity, Dietary Administration - Rat; OPPTS 870.3100 (rodent).

DP BARCODE: D281584 SUBMISSION CODE: S612009
P.C. CODE: not provided

TEST MATERIAL (PURITY): Silver, zinc, and ammonia complex substitution type A zeolite (purity not provided)

SYNONYMS: AZN zeolite; zeomic

CITATION: Hirasawa, F., Takizawa, Y., Tsunoda, H., Uesugi, S., Fjuii, M. (1994) Studies on silver, zinc and ammonia complex substitution type A zeolite from the perspective of public health studies I: subchronic toxicity test. Japanese Journal of Food Chemistry, Vol.1(1). MRID No. 455811-06.

SPONSOR: Sinanen Company Ltd.
4-22, Kaigan 1-Chome, Minato-ku
Tokyo 105, Japan

EXECUTIVE SUMMARY

In a subchronic toxicity study (MRID 45581106), silver, zinc and ammonia complex substitution type A zeolite (AZN zeolite) (purity not provided) was administered to 100 four-week old Fischer-344 rats (ten/sex/group) in the diet for 13 weeks. Rats were divided into five groups: control, AZN zeolite 1.25% feed mixture, AZN zeolite 2.5% feed mixture, AZN zeolite 5.0% feed mixture, and satellite (group to which a four-week recovery period was provided following administration of AZN zeolite 5.0% feed mixture).

Clinical signs of toxicity included poor skin complexion, reduced spontaneous movement, emaciation, and tremors, beginning during weeks 7-8. Two premature deaths occurred during weeks 11 and 13 of the 13-week administration period, and six premature deaths occurred during weeks 14 and 15 of the four-week recovery period. Five of the six premature deaths that occurred during the recovery period resulted from atrial thrombosis, which the study authors attributed to the toxicity of the test material. All premature deaths involved animals that were administered 5.0% AZN zeolite feed mixture. Treatment-related effects were observed in body weight gain, food consumption, hematology, clinical chemistry, and histopathology. Following discontinuation of administration of AZN zeolite, body weight gain and food consumption improved. Significant dose-dependent patterns were observed for many hematological and clinical chemistry parameters. Histopathology revealed pigmentation of numerous organs and fibrosis and atrophy of the pancreas. **The study authors did not determine NOELs or LOELs. Our reviewers concluded that the LOEL is 1.25% based on: significantly lower values for body weight gain and food consumption, dose-dependent patterns for many hematological and clinical**

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chemistry components, pigmentation of numerous organs, and other non-tumorigenic changes. Our reviewers were unable to convert this value to mg/kg-day because actual doses were not provided and concentrations in the diet could not be converted to doses.

This subchronic toxicity study is classified as **unacceptable** because evaluation of certain parameters (e.g., functional observations, ophthalmology, organ weights) necessary for the adequate assessment of subchronic toxicity was not conducted, and evaluation of other parameters (e.g., histopathology) was incomplete. Furthermore, no stability, homogeneity, or concentration analyses were reported for the test diets, which prevents an adequate assessment of the amount of test material that the animals received during the course of the study and therefore assignment of NOAEL/LOAEL values. Therefore, this study does not satisfy the guideline requirements for a subchronic oral study (OPPTS 870.3100) in rodents. The study is **not upgradeable** as discussed in the study deficiencies section of this evaluation report.

COMPLIANCE

Signed and dated Good Laboratory Practice (GLP) and No Data Confidentiality Claims statements were provided by the submitter. However, the GLP statement indicated that the submitter did not know whether the study had been conducted in accordance with EPA GLP Standards (40 CFR Part 160). The study report stated that the 3-month subchronic toxicity test via oral administration in rats was carried out according to OECD guidelines for testing of chemicals (Ministry of International Trade and Industry, Basic Industry Bureau, Chemical Safety Section, 1985).

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Silver, zinc and ammonia complex substitution type A zeolite (AZN zeolite)
   Description: Fine, white powder
   Lot/Batch #: Not provided
   Purity: Not provided; contains 2-2.5% silver (Ag), 11-13% zinc (Zn), and 2-3% ammonia (HNO_3).
   Stability of compound: No information provided
   CAS #: Not provided

2. Vehicle and/or positive control: None used; administered in feed

3. Test animals
   Species: Rat
   Strain: Fischer-244
   Age and weight at study initiation: Animals were approximately 4 weeks old when dosing began. Figure 1 of the study report showed that mean body weights for male and female dose groups at study initiation were approximately 125-130 grams and 105-110 grams, respectively. The mean body weights for male and female dose groups were estimated from the graphs in Figure 1 because actual mean body weight values were not included in the study report.

Source: Shizouka Experimental Animals Cooperative Association
Housing: 5 per cage
Diet: Commercial mouse and rat feed (Oriental Yeast Co., Ltd., MF), ground, mixed with test article, and repelletized provided *ad libitum*.
Water: Tap water provided *ad libitum*.
Environmental conditions: Temperature: 23 ± 1°C (target)
Humidity: 50 ± 10% (target)
Air changes: No information provided
Photo period: 14 hours light/10 hours dark cycle
Acclimation period: One week

B. STUDY DESIGN

1. In life dates: No information provided

2. Animal assignment

Animals were divided into 5 test groups with 10 animals each (5/sex/cage). Randomization procedures were not described; however, the study authors indicated that animal assignment was conducted in a manner that avoided body weight difference among groups as much as possible. The study authors did not indicate how the animals were uniquely identified.

Table 1. Study Design

Test Group	Conc. in Diet [a]	Male	Female
Control	0.0%	5	5
1.25% Group	1.25%	5	5
2.5% Group	2.5%	5	5
5.0% Group	5.0%	5	5
Satellite [b]	5.0%	5	5

a Concentrations in the test diet could not be converted to doses (mg/kg-day) due to the absence of body weight and food consumption data. This information appears to have been calculated by the study authors (see p. 6 under Statistical Analysis), but it was not provided in the study report.

b Satellite group was given four-week recovery period following administration of 5.0% AZN zeolite feed mixture during the 13-week study.

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3. Diet preparation and analysis

The AZN zeolite feed was prepared by grinding commercial feed, mixing the appropriate amounts of test material, and pelletizing the mixture again. The study authors did not indicate the frequency of preparation or describe the storage conditions of the feed mixture. Also, they did not indicate whether homogeneity, stability, or concentration analyses were performed on the test diets. In Table 2 of the study report (p. 000416), the study authors included information about the nutritional composition and contamination of the solid feed used. The study authors noted that no significant contamination was observed in the test material or the commercial feed.

4. Statistics

Group means and standard deviations for body weight and food consumption were calculated based on individual animal body weight and per cage food consumption data, respectively. The study authors indicated that analysis of variance (ANOVA) was used to assess body weight and food consumption data. While ANOVA is useful for determining whether there are statistically significant differences among study groups, it is not appropriate for assessing differences between individual treatment groups and the control group. There is no indication that another statistical method was used for pairwise comparisons. Furthermore, there is no indication that the data were analyzed for normality or homogeneity, which are standard assumptions of the ANOVA.

Group means and standard deviations were calculated for organ weight, hematological, and serum biochemical data based on individual animal data. The data were assessed for normality using Smirnoff's abnormality test; however, there is no indication that the data were analyzed for homogeneity. Pairwise comparisons between treatment groups and the control group were conducted using a t-test (specific type not indicated). While this test is appropriate for making pairwise comparisons, more powerful statistical tests are available (e.g., Williams' test or Dunnett's test).

The Mantel-extension method was used to calculate differences in weekly survival rates between the control group and each treatment group. The Mantel-extension method and Fischer's Exact test were used to evaluate data on the incidences of histological changes.

C. METHODS

1. Observations

Gross observations of animals were performed daily for signs of toxicity and mortality. Details of these observations were not provided by the study authors.

2. Body weight

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Animals were weighed once during the week prior to initiation of treatment and each week thereafter.

3. Food consumption, water consumption, and compound intake

Food consumption was recorded once at the initiation of treatment and each week thereafter. Weekly diet consumption was shown as mean per cage and reported in g food/day. Water consumption was not quantified. Compound intake reportedly was calculated using weekly mean body weight and food consumption data; however, these values were not provided in the study report.

4. Ophthalmoscopic examination

Ophthalmoscopic examinations were not conducted.

5. Clinical laboratory tests

Blood sampling for hematology and clinical chemistry was conducted for all animals. The animals were fasted overnight and anesthetized with ether. Initially, 1 mL of blood was taken from the subclavian venous plexus for hematology, and an additional 3-4 mL of blood was taken for serum biochemistry tests.

a. Hematology

Analyses were conducted for the CHECKED (X) hematology parameters. The study authors also noted that several samples were randomly selected from each group to prepare smear test slides and that morphological configurations of blood cells were observed using a microscope.

X	Hematocrit (HCT)*		Differential leukocyte count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB conc. (MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)*
X	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Activated partial thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for subchronic studies based on OPPTS Health Effects Test Guidelines 870.3100.

b. Clinical chemistry

Analyses were conducted for the CHECKED (X) serum biochemistry parameters. The study authors also noted that high density lipoprotein

cholesterol (HDL-C) was assayed using the enzymatic method (Eiken Kit) and that low density lipoprotein cholesterol (LDL-C) was calculated from the difference between total cholesterol (TC) and HDL-C. In addition, serum Ag and Zn were quantified by atomic absorption (Nippon Jarrel Ash, AA-780).

	ELECTROLYTES		OTHER
X	Calcium	X	Albumin*
	Chloride	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus	X	Total cholesterol*
	Potassium*		Globulins
	Sodium*		Glucose*
	ENZYMES	X	Total bilirubin
		X	Total serum protein (TP)*
X	Alkaline phosphatase (ALK)*	X	Triglycerides
	Cholinesterase (ChE)		Serum protein electrophores
	Creatine phosphokinase		
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Recommended for subchronic studies based on OPPTS Health Effects Test Guidelines 870.3100. These guidelines suggest the measurement of more than two hepatic enzymes. In addition to the enzymes indicated in the table, the guidelines provide two other suggestions (i.e., sorbitol dehydrogenase and gamma glutamyl transpeptidase). Other measurements that may be indicated for a specific study include bile acids, calcium, phosphorus, fasting triglycerides, hormones, methemoglobin, and cholinesterases.

6. Urinalysis

Urinalyses were not conducted. Such tests are not recommended for subchronic studies based on OPPTS Health Effects Test Guidelines 870.3100.

7. Sacrifice and histopathology

The method of sacrifice was not clear. According to the report (page 5), at the end of the treatment period or the treatment + recovery period, "animals were fasted overnight, anesthetized with ether..." And blood sampled form the subclavian venous plexus for hematology and serum biochemistry measurements. Organs were then harvested following this.

It is true that the sequence of events is not adequately described. However, it appears that ether anesthesia was followed by exsanguination and then organs removed. There is no indication that the animals were subjected to gross necropsy examination.

The statistical methods section indicates that data on organ weights were collected; however, there is no mention of organ weight determinations or findings elsewhere in the study. The CHECKED (X) tissues and organs were collected for histopathological examination. Tissues were fixed in a 10% formalin solution, stained with hematoxylin and eosin, and submitted for histopathology. The tissues indicated in the list were microscopically examined in all animals using an optical microscope.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta*	X	Brain (3 levels)*+
X	Salivary glands*	X	Heart*		Periph. nerve (sciatic or tibial)*
X	Esophagus*		Bone marrow*		Spinal cord (3 levels)T
X	Stomach*	X	Lymph nodes*		Pituitary*
X	Duodenum*	X	Spleen*	X	Eyes (retina & optic nerve)T
X	Jejunum*	X	Thymus*		
X	Ileum*				GLANDULAR
X	Cecum*			X	Adrenal gland(s)*
X	Colon*		UROGENITAL		Lacrimal gland T
	Rectum*			X	Mammary gland (female)T
X	Liver*+	X	Kidneys*+		Parathyroid*
	Gall bladder*	X	Urinary bladder*	X	Thyroid*
X	Pancreas*	X	Testes*+		
			Epididymides		OTHER
	RESPIRATORY		Prostate		
		X	Seminal vesicle(s)	X	Bone
X	Trachea*	X	Ovaries	X	Skeletal muscle
X	Lungs*	X	Uterus*	X	Skin
	Nose				All gross lesions and masses*
	Pharynx				
	Larynx				

* Recommended for subchronic studies based on OPPTS Health Effects Test Guidelines 870.3100.
+ Organ weight required in subchronic studies based on OPPTS Health Effects Test Guidelines 870.3100.
T Organ weight required only when toxicity or target organ.

II. RESULTS

A. Observations

1. Toxicity

The study authors noted that clinical signs of toxicity were observed in both males and females in the 2.5% and 5.0% groups beginning in weeks 3-4 of administration. During weeks 3-4, a reduction in hair cast wetting was observed. Poor skin complexion, reduced spontaneous movement, emaciation, and tremors began to appear during weeks 7-8 in the 2.5% and 5.0% dose groups.

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2. Mortality

Mortality data are summarized in Table 3 of the study. Premature deaths occurred among males in the 5.0% group and both males and females in the satellite group. In the male 5.0% group, one premature death occurred during week 11 and two occurred during week 13. In the male satellite group, one premature death occurred during week 14 (i.e., week 1 of the recovery period). In the female satellite group, three premature deaths occurred during week 14 and two occurred during week 15 (i.e., week 2 of the recovery period). According to the study authors, the occurrence of deaths in the satellite group during the recovery period is indicative of a delayed recovery from the effects of the test article administration. All animals in the control, 1.25%, and 2.5% groups survived to terminal sacrifice.

B. Body weight and weight gain

In Figure 1 of the study report, the study authors provided growth curves for males and females in all groups, but actual values for body weight and body weight gain were not included. Overall, the study authors noted that males in the 1.25%, 2.5%, and 5.0% groups and females in the 2.5% and 5.0% groups showed significantly lower weekly body weight gains compared to those of the controls. ANOVA reportedly confirmed that growth in these groups was significantly lower compared to that of the controls throughout the study period. Our reviewers note, however, that ANOVA is not intended for pairwise comparisons of each treatment group to the control group.

The study authors stated that at 3 months of drug administration, growth rates showed a trend of dose-dependent decreasing effects. The growth rate among males in the 5.0% group was reported to be 114.7%, which was only 40% of the control group rate. The growth rate among females in the 5.0% group was reported to be 116.9%, which was only 60% of the control group rate. For the satellite group, following discontinuation of administration of the AZN zeolite feed mixture, recovery of body weight gain was observed in both male and female animals, beginning in the third week of the four-week recovery period. The study authors stated that male animals in the satellite group recovered to 181.5% of the control group, and female animals in the satellite group recovered to 163.8% of the control group.

C. Food consumption

In Figure 2 of the study report, the study authors graphically showed the change of food consumption for males and females in all groups, but actual values for food consumption were not included. Overall, the study authors noted that decreases in food consumption were observed in both males and females in the 2.5% and 5.0% groups. ANOVA reportedly confirmed that food consumption was significantly different in these groups compared to controls.

The study authors stated that after 3 months of treatment, weekly food consumption in the 5.0% group was observed to be 6.0 grams in males and 6.45 grams in females. These

No

values represented decreases of 64.3% and 36.5%, respectively, compared to corresponding control values. The study authors also noted that food consumption began to increase in the satellite group during the third week of the four-week recovery period.

D. Clinical laboratory tests

1. Hematology

The hematology findings are summarized in Table 4 of the study report. In the 1.25%, 2.5%, and 5.0% groups, the animals showed significant reductions in RBC count, HGB, HCT, and MCH, compared with the control group. The MCV levels were significantly decreased in males at 1.25% and 2.5% and in females at all doses. The MCHC was significantly decreased at 2.5% in males and 1.25% and 5.0% in females. The study authors stated that RBC count, HGB, and HCT also showed dose-dependent decreasing trends. Following the four-week recovery period, however, these three parameters improved in the satellite groups to levels near those of the control groups. The study authors noted that heterotypic erythrocytes in the 2.5% group and nucleated erythroblasts in the 5.0% group were observed on smear test slides.

WBC counts in both males and females in the 5.0% group were significantly higher compared to the controls while those in females at 2.5% were significantly lower than controls. In the satellite groups, following the four-week recovery period, WBC counts in males were significantly decreased compared to controls; values for females were comparable to controls.

The study authors stated that the number of platelets were higher in both males and females in all treatment groups than in the controls. Significant increases were observed in males in the 1.25%, 2.5%, and satellite groups and in females in the 2.5%, 5.0%, and satellite groups. The observed values were significantly higher in the satellite groups than in the controls despite discontinuation of test material administration during the four-week recovery period.

2. Clinical chemistry

The clinical chemistry findings are summarized in Table 5 of the study report. Significantly lower values were observed for total protein, albumin, HDL-C/TC, and blood creatinine in both male and female animals in all groups compared with the control group. These parameters showed a dose-dependent decreasing trend in all groups, including the satellite groups. The study authors noted that the values for total protein, albumin, and HDL-C/TC increased in the satellite groups following the four-week recovery period; however, significantly lower values compared with the control groups were still observed. Creatinine continued to decrease even after discontinuation of administration of AZN zeolite.

In contrast, TC, alkaline phosphatase, LDL-C, and LDL-C/HDL-C showed dose-dependent increasing trends that were significantly higher in the treatment

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groups than values observed for the controls. Although the values for alkaline phosphatase, LDL-C, and LDL-C/HDL-C decreased in the satellite groups following the four-week recovery period, significantly higher values compared with the control groups were still observed. Also, TC continued to increase in the satellite groups even after discontinuation of administration of AZN zeolite.

E. Histopathology

In Table 6 of the study report, the study authors summarized the incidences of histopathological changes. Dark gray pigmentation was observed in the pancreas, liver, kidneys, stomach, and lymph nodes of treated males and females as shown below in Table 2.

Table 2. Incidence of Pigmentation in Organs Examined

Group		Pancreas	Liver	Kidneys	Stomach	Lymph Nodes
Males	Controls	0/10	0/10	0/10	0/10	0/10
	1.25%	10/10	0/10	0/10	3/10	0/10
	2.5%	10/10	10/10	0/10	9/10	5/10
	5.0%	9/10	9/10	0/10	9/10	7/10
	Satellite	10/10	8/10	1/10	9/10	6/10
Females	Controls	0/10	0/10	0/10	0/10	0/10
	1.25%	10/10	10/10	0/10	10/10	2/10
	2.5%	10/10	10/10	0/10	10/10	5/10
	5.0%	9/10	10/10	2/10	10/10	9/10
	Satellite	9/10	9/10	1/10	7/10	5/10

Even after the four-week recovery period, no disappearance of pigmentation was observed. The study authors noted, however, that the gray color intensity appeared to decrease in the stomach and lymph nodes for the satellite groups.

The study authors reported the occurrences of fibrosis and atrophy of the pancreas at a rate of 90-100 percent in the 5.0% group. Fibrosis and atrophy of the pancreas appeared to be treatment-related. Following discontinuation of administration of AZN zeolite, fibrosis significantly improved in the male and female satellite groups. Atrophy did not improve as the same percentage of animals affected at the high dose remained so following the recovery period.

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Atrial thrombosis was observed in two males and four females in the satellite group. The study authors noted that this disease was the direct cause of death for five of these six animals during the four-week recovery period. Hypercardia was observed in three males in the 1.25% group, two males in the satellite group, and four females in the satellite group.

There were no tumorigenic changes in any animals.

III. DISCUSSION

A. Overview

The study authors noted treatment-related changes in a number of parameters. Males and females in all treatment groups, except females in the 1.25% group, showed significantly lower body weight gain compared to the controls. Food consumption values for male and female animals in the 2.5% and 5.0% groups were significantly lower than the controls. Following discontinuation of administration of AZN zeolite, however, body weight gain and food consumption began to increase to levels near those observed in the controls. The study authors noted that suppression in food consumption and growth retardation are phenomena frequently observed in animals that have consumed large amounts of Ag or Zn, which are components of AZN zeolite. Significant dose-dependent patterns were observed for many hematological and clinical chemistry components. Following the four-week recovery period, the erythrocyte-related reductions had improved to 70-80% of the control group. High levels of alkaline phosphatase, LDL-C, and LDL-C/HDL-C were still observed following the recovery period. The study authors concluded that these findings reflect a long mean residual time of Zn in the body based on current literature and the fact that serum Zn levels in male and female animals remained high even after administration of the test material was discontinued.

The study authors stated that no tumorigenic changes were observed during the 3-month administration period. Non-tumorigenic changes consisted of: atrial thrombosis, hypercardia, pancreatic fibrosis and atrophy, and pigmentation of numerous organs. Fibrosis and atrophy of the pancreas were presumed to be histological changes reflecting the toxicity of Zn. Atrial thrombosis occurred during the recovery period and was the direct cause of death for five satellite group animals; the study authors noted that this condition may have been due to: (1) insoluble Ag remaining in tissues for a long time, (2) continued disturbances to epithelial cells by Ag even after discontinuation of administration, or (3) the high residuality of Zn and delayed recovery of serum lipid and platelet functions.

The study authors concluded that most of the treatment-related changes were due to mutual activities of Zn and Ag derived from the test material. Most of these effects were dose-dependent, and the majority of symptoms were alleviated by discontinuation of administration of AZN zeolite. Our reviewers agree with the study authors' conclusions,

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but note that this study was not adequate for complete evaluation of subchronic toxicity (see *Study deficiencies* below).

B. Study deficiencies

Because this study was not conducted for regulatory purposes, major deficiencies exist when compared to EPA's guidelines for subchronic oral toxicity studies (i.e., OPPTS Health Effects Test Guidelines 870.3100). For example, no details were provided on the daily clinical observations conducted throughout the study. Therefore, our reviewers could not determine whether all of the necessary parameters were evaluated (e.g., assessment of motor activity, grip strength, or sensory reaction to stimuli). In addition, the toxicological endpoints evaluated were limited and incomplete. Ophthalmological examinations and gross necropsies were not conducted, and no organs submitted for histopathology were weighed. Standard parameters for hematology (e.g., blood clotting and differential leukocyte count), clinical chemistry (e.g., potassium, sodium, and glucose), and histopathology (e.g., gall bladder, nose, pharynx, larynx, and pituitary) were omitted. Because these deviations would prevent an adequate assessment of subchronic toxicity, this study is classified as unacceptable. The study is not upgradeable because these deficiencies cannot be corrected after completion of the study.

Because this study was presented as a published journal article, it is likely that page limitations restricted the amount and detail of the information provided. Most notably, there is no indication as to whether stability, homogeneity, or concentration analyses were conducted on the diet formulations, and the purity of the test material was not provided. These deficiencies are particularly significant because the amount of test material that the animals received is uncertain as a result. Another major reporting deficiency is the lack of group summary data for clinical signs, body weight, and food consumption as well as individual animal data for all parameters. As a result, our reviewers could not verify any of the study findings. In addition, test article intake reportedly was calculated from body weight and food consumption data, but these values were not presented. Our reviewers could not calculate doses in mg/kg-day in the absence of the group summary data on body weight and food consumption. The information that is lacking probably could be provided by the testing laboratory or the sponsor of the study.

It is of interest that some of the adverse effects observed in this study seem to be unique to administration of the silver zeolite as AZN zeolite. For example, atrial thrombosis, hypercardia, pancreatic fibrosis and atrophy, and pigmentation of numerous organs (other than the pancreas) have not been observed in other toxicity studies conducted with silver zeolite. The observation that some of the serum chemistry parameters, notably alkaline phosphatase, LDL-C, and LDL-C/HDL-C were still increased following the recovery period is also a novel finding with regard to silver zeolite toxicity. The study authors concluded that these findings reflect a long mean residual time of Zn in the body (reported as long as 262 hr) and was corroborated by serum Zn levels in male and female animals that remained high even after administration of the test material was discontinued. Other studies with silver zinc zeolite have not investigated this type of effect. Certainly, the effects from administration of AZN zeolite merit further investigation to determine the dose at which these effects cease to appear. The cardiac

effects are particularly of concern as they were observed primarily in the satellite female animals which had undergone a 4-week recovery period. The suggestion that hypercardia may be based upon the potential of silver to produce thickening of epithelium, and that atrial thrombosis may be based upon insoluble complexes formed by deposited silver as well as toxicity of zinc on serum lipid and platelet function, deserves further investigation.

DATA EVALUATION REPORT

ZEOMIC

Study Type: Combined Chronic Toxicity/Carcinogenicity (Mice and Rats)

Prepared for

Antimicrobial Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by

ICF Consulting Group
9300 Lee Highway
Fairfax, VA 22031

Under Subcontract to:

Versar
6850 Versar Center
P.O. Box 1549
Springfield, VA 22151

Principal Reviewer	Robyn Blain, Ph.D.	Date June 3, 2002
Independent Reviewer	Chad Sandusky, Ph.D.	Date June 4, 2002
Project Manager	Kara Altshuler, Ph.D.	Date 6/4/02
Versar Program Manager	Linda Phillips, Ph.D.	Date 6/7/02

Contract Number:68-W-01-036
Work Assignment No.:0248.2000.002.02
EPA Work Assignment Manager: Cletis Mixon, Ph.D.

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EPA Reviewer: Timothy F. McMahon, Ph.D. _____ Date: 8-28-02
Senior Toxicologist/Antimicrobials Division (7510C)

DATA EVALUATION RECORD

STUDY TYPE: Combined Chronic Toxicity/Carcinogenicity, Dietary Administration-Mice and Rats; OPPTS 870.4300

DP BARCODE: D281584 SUBMISSION CODE: S612009
P.C. CODE: not provided

TEST MATERIAL (PURITY): The first report lists the compound as Zeomic AJ 10N (contains 2.3% Ag, 12.5% Zn, and 2.5% NH_4) while the second report lists the compound as silver-, zinc-, ammonia-complex substituted zeolite A or silver complex zeolite with 2.5±0.5% (mean 2.6%) Ag, 14.5±1.5% (mean 14.5%) Zn, and 2.5±0.5% (mean 2.5%) NH_4. The second report states that the compound was produced and supplied by Shinagawa Fuel Co.

SYNONYMS: antibiotic zeolite

CITATION: **First report**: Takizawa, Y. (1994). Combined Chronic Toxicity/Carcinogenicity Study of Zeomic in Mice and Rats. Depatment of Public Hygiene, Akita University, School of Medicine. (Japan). April 25, 1994. MRID 45581107. Unpublished.

Second report: Takizawa, Y., Hirasawa, F., Fujii, M., et al. (1995). A Study on Oral Chronic Toxicity and Carcinogenicity of Silver-, Zinc-, Ammonia-Complex-Substituted Zeolite. (Japan). Accepted on March 20, 1995. Unpublished.

SPONSOR: Sinanen Company Ltd.
4-22, Kaigan 1-Chome, Minato-ku
Tokyo 105, Japan

EXECUTIVE SUMMARY

Two studies (MRID 4551107) are reviewed in this DER, both using Zeomic, containing 2.3% Ag, 12.5% Zn and 2.5% NH4.

In the first study, a combined chronic toxicity/carcinogenicity study, B6C3F1 (SPF) mice (50 sex/dose plus additional animals in satellite groups for interim examination) were fed Zeomic at dose levels of 0, 0.1%, 0.3% or 0.9% for 24 months. These doses were calculated by this reviewer to correspond to 106, 340 and 1015 mg/kg bw/day for males based on data provided on nominal concentrations, food intake and body weights. A significant portion of the female data were missing and similar calculations for dose conversions were not possible. In the chronic portion of this study, the most consistent and

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sensitive effects were observed on hematological parameters at the 0.3% dietary level (decreased RBC, HBG, HCT and MCV in both sexes, although females appeared slightly more sensitive). The NOAEL for these effects was 0.1% for females (calculated by this reviewer to be 106 mg/kg bw/day for males) and the LOAEL was 0.3% (calculated by this reviewer to be 340 mg/kg bw/day for males). There were also effects on organ weights, i.e., increased pancreatic weights in males at 24 months, the NOEL being 0.1% (106 mg/kg bw/day) and the LOAEL = 0.3% (340 mg/kg bw/day) and various non-neoplastic histopathological observations, e.g., enlargement of Langerhans' island in males and ovarian cysts in females. These histopathological effects are difficult to interpret in that all animals that died on study and those at terminal sacrifice were combined into one summary table (see relevant section of DER foradditional information). **The NOAEL for chronic toxicity in mice was determined to be 0.1% (106 mg/kg bw/day) and the LOAEL was 0.3% (340 mg/kg bw/day) based on the above changes in hematology and organ weight changes.**

The chronic toxicity study in mice is classified **unacceptable** and does not satisfy the guideline requirement for a chronic oral toxicity study in mice (OPPTS 870.4300). There were several deficiencies observed in this study, including no clinical chemistry determinations, no urinalyses, no opthalmological exams, missing or under-reporting of data (e.g., missing body weights for females for weeks 0-17 and 40-70), and some missing organs for histopathological exam. These data would be required in order to consider the study acceptable for regulatory purposes. Additional details may be found in the deficiency section of the DER as well as in "Appendix A, Data Validation."

In the second portion of this study, the carcinogenic potential of Zeomic in mice was evaluated. Based on the data as presented, there appears to be no increase in tumors in this study. However, the tumor data (as well as the non-tumor data) are summarized by combining all animals that died on study (or sacrificed moribund) with those that were sacrificed at termination. Thus, without a separate analysis of these two groups, a final assessment of the carcinogenic potential of Zeomic in this study is difficult. Although there was a lack of significant toxic effect at the high dose in mice, this dose appears to approximate a limit dose (1000 mg/kg). Thus, the data on carcinogenicity in mice may be useful in the future for determination of the carcinogenic potential of Zeomic.

This carcinogenicity study in mice is **unacceptable** in that it was not conducted under GLPs. The study does not currently satisfy the guideline requirement for a carcinogenicity study in mice (OPPTS 870.4300). However, in that the males were exposed to the approximate Limit Dose, this study may provide useful information on the potential carcinogenicity of Zeomic in mice. If the tumor data are presented in proper format, e.g., with animals that died on study summarized by time of death separately from those that were sacrificed at termination, the study would be more useful in this respect. Also deficiencies in under-reporting listed in "Appendix A, Data Validation" should be addressed to the extent possible, e.g., clarification of exactly which tissues underwent a histopathological examination. Clear dose conversions from % to mg/kg bw/day should also be provided by the submitter. Pending receipt and review of this additional information, the final utility of these data in the evaluation of carcinogenicity remains uncertain.

In the second study, also a combined chronic toxicity/carcinogenicity study, Fisher 344 (SPF) rats (50 sex/dose in the main study group plus additional animals in satellite groups for interim examination)

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were fed Zeomic at dose levels of 0, 0.01%, 0.03%, 0.1% or 0.3% for 24 months. These doses were calculated by this reviewer, based on nominal concentrations in the diet, food intake and body weights to be 4.1, 12.2, 39.5 and 121 mg/kg bw/day for males and 4.1, 12.5, 41.1 and 125 mg/kg bw/day for females.

In the chronic portion of this study, consistent effects were again observed on hematological parameters (decreased HBG, HCT, MHC and MCHC in both males females at 24 months, although females again appeared more sensitive). There were other biochemical effects noted in this study (e.g., increased ALT and ALP), but they occurred at higher doses than those that affected the hematological parameters and there were no corresponding histopathological changes in the liver. **The NOAEL for chronic toxicity this study was 0.03% or 12.5 mg/kg bw/day (based on changes in hematological parameters in female rats) and the LOAEL = 0.1% or 41.1 mg/kg bw/day.**

The chronic portion of the rat study is **unacceptable** since it was not conducted under GLPs and other deficiencies were noted, such as no urinalysis, no ophthalmological exams, combining histopathology for non-neoplastic and neoplastic lesions from all animals that died on study with those sacrificed at termination, and significant portions of missing or under-reported data as outlined in "Appendix A, Data Validation".

In the second portion of this study, the carcinogenic potential of Zeomic in rats was evaluated. Based on the data as presented, there appears to be no increase in tumors in this study. There was an increase noted in leukemia (females) and endometrial polyps which were statistically significant for trend and/or by pair-wise comparison. These effects were dismissed in the report as being within the range of historical controls. These historical control data were not provided for review. In addition, the tumor data (as well as the non-tumor data) are summarized by combining all animals that died on study (or sacrificed moribund) with those that were sacrificed at termination. Thus, without a separate analysis of these two groups, a final assessment of the carcinogenic potential of Zeomic in this study is difficult. Finally, there was not sufficient toxicity observed to consider that the high dose was adequate for carcinogenicity testing.

This carcinogenicity study in rats is **unacceptable** in that it was not conducted under GLPs. The carcinogenicity data may be viewed as supplementary but the study does not satisfy the guideline requirement for a carcinogenicity study in rats (OPPTS 870.4300). There are no data to support that the top dose represented or even approximated an adequate dose for carcinogenicity testing. Survival was not adversely affected at the high dose nor were there any effects that could be supported as toxicologically significant.

COMPLIANCE

Signed and dated GLP statement and No Data Confidentiality Claims were provided. The GLP statement claims that the study did not meet the requirements of 40 CFR Part 160. There was no study director or quality assurance for the testing. Therefore, this study was not conducted under GLP standard. The study report claims to have followed the guidelines of EPA number 870.4300, OECD number 453, and EC Directive 87/302/EEC.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test Material: Zeomic AJ 10 N (silver-, zinc-, ammonia-complex substituted zeolite A)*
   Description: A tasteless and odorless crystalline powder.
   Lot number: 80090 (none provided)*
   Purity: Zeolite with 2.3% Ag, 12.5% Zn and 2.5% NH_4 . (2.6% Ag, 14.5% Zn, and 2.5% NH_4)*. All other contaminants examined were below detection.
   Stability of compound: No information provided.
   CAS # : Not provided.
   * information in parentheses was provided in the second report and was inconsistent with the first report.

2. Vehicle control: Diet without test compound.

3. Test animals
   a) Species: Mouse
   Strain: B6C3F1 (SPF)
   Source: Japan SLC, Ltd.

   Age and weight at study initiation: Mice were 5 weeks old at the beginning of the study. Male mice had a body weight range of 18.1-25.1 grams, female mice had a body weight range of 16.2-20.1 grams.

   Housing: 5 mice of the same sex per cage.
   Diet: Solid MF diet with or without Zeomic added was provided *ad libitum*[1].
   Water: Tap water provided *ad libitum*.[2]
   Environmental conditions: Temperature: 23±1°C
   Humidity: 50±10%
   Air changes: not specified
   Photoperiod: 14 hours light/10 hours dark cycle
   Acclimation period: 1 week

   b) Species: Rat
   Strain: Fischer 344 (SPF)
   Source: Japan SLC, Ltd.

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[1]The study authors provide the composition of the diet including contaminants in Table 4. The study authors note that the presence of the test compound does not change the nutritional properties of the diet.

[2]The study authors do not mention analysis of the water provided or possible contaminants in the water.

Age and weight at study initiation: Rats were 5 weeks old at the beginning of the study. Male rats had a body weight range of 73.7-108.9 grams and female rats had a body weight range of 69.7-97.0 grams at the beginning of dosing.

Housing: 5 rats of the same sex per cage.

Diet: Solid MF diet with or without Zeomic added was provided *ad libitum*[3].
Water: Tap water provided *ad libitum*.[4]
Environmental conditions: Temperature: 23±1°C
Humidity: 50±10%
Air changes: not specified
Photoperiod: 14 hours light/10 hours dark cycle
Acclimation period: 1 week

B. STUDY DESIGN

1. In life dates

Male mice
Start: June 22, 1989
End: June 3-6, 1991

Female mice
Start: June 23, 1989
End: June 17-20, 1991

Male rats
Start: September 20, 1990
End: September 28-October 2, 1992

Female rats
Start: September 21, 1990
End: October 5-9, 1992

2. Animal assignment

Mice:

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[3]The study authors provide the composition of the diet including contaminants in Table 4. The study authors note that the presence of the test compound does not change the nutritional properties of the diet.

[4]The study authors do not mention analysis of the water provided or possible contaminants in the water.

Animals were housed 5 per cage so that there were no statistically significant changes in mean body weight between the cages or between the groups. The assignment was based on dividing the animals into 5 groups according to body weight, 1 animal from each group was placed in a cage. The study authors do not mention a random assignment, they made arrangements so that the mean body weights were the same among the groups. The study authors do not specify how the animals were uniquely identified. Animals were assigned to the groups as detailed in Table 1.

Table 1. Study Design:Mice

Test Group	Conc. In Diet	Male				Female			
		3 mo.	6 mo.	12 mo.	24 mo.	3 mo.	6 mo.	12 mo.	24 mo.
Control	0%	5	10	10	50	5	10	10	50
Low	0.1%	5	10	10	50	5	10	10	50
Intermediate	0.3%	5	10	10	50	5	10	10	50
High	0.9%	5	10	10	50	5	10	10	50

Rats:

Animals were housed 5 per cage so that there were no statistically significant changes in mean body weight between the cages or between the groups. The assignment was based on dividing the animals into 5 groups according to body weight, 1 animal from each group was placed in a cage. The study authors do not mention a random assignment, they made arrangements so that the mean body weights were the same among the groups. The study authors do not specify how the animals were uniquely identified. Animals were assigned to the groups as detailed in Table 2.

Table 2. Study Design:Rats

Test Group	Conc. In Diet	Males			Females		
		6 mo.	12 mo.	24 mo.	6 mo.	12 mo.	24 mo.
Control	0%	10	10	50	10	10	50
Low	0.01%	10	10	50	10	10	50
Intermediate I	0.03%	10	10	50	10	10	50
Intermediate II	0.1%	10	10	50	10	10	50
High	0.3%	10	10	50	10	10	50

3. Dose-selection Rationale

Mice:

The study authors state that "A 3-month sub-chronic toxicity study of the antibiotic zeolite of Shinanen Co., Ltd. (Zeomic) performed in accordance with the OECD guidelines, which is an international testing standard, revealed its effects at middle and high doses, and valuable information for evaluation of long-term chronic toxicity has been obtained." Our reviewers were not provided details of the subchronic studies. Therefore, the rational for dose selection cannot be evaluated.

Rats:

The study authors state that "A 3-month sub-chronic toxicity study of the antibiotic zeolite of Shinanen Co., Ltd. (Zeomic) performed in accordance with the OECD guidelines, which is an international testing standard, revealed its effects at middle and high doses, and valuable information for evaluation of long-term chronic toxicity has been obtained." Our reviewers were not provided details of the subchronic studies. Therefore, the rational for dose selection cannot be evaluated

4. Diet preparation and analysis

Mice and Rats:

Compound from the same lot (80090) was maintained in a tightly closed container and stored at room temperature. When the diet was prepared, the appropriate amount was removed. The study authors claim in the first report that the diets were prepared once every 3-4 months by the Oriental Yeast Co., Ltd. from a solid (pellet) stock food for mice and rats (MF) produced by Oriental Yeast Co., Ltd. The second report claims that the test substance was mixed into the commercial diet and adjusted to the correct percentages before feeding and that the diet was adjusted once in 3-4 months. The diet was prepared so that the internal

percentage of Zeomic was at the target concentrations. A control diet was prepared in the same manner without the test substance. The diet was stored at 4°C until use. The study authors do not mention any analysis performed to verify that the target concentrations were achieved. Therefore, any concentrations used were nominal concentrations not analytical concentrations. The study authors do specify that the Zeomic did not change the nutritional properties of the diet or any contaminant levels in the diet.

5. Statistics

Mice:

Weekly mean body weights and food consumption were compared using a t-test. A two-way ANOVA was used to detect differences in serial changes in body weight. Organ weight and hematology parameters were converted to logarithms and examined by Gruibbs-Smirnov rejection test for the detection of abnormal values. Abnormal values were excluded from subsequent analysis. A t-test was performed on remaining data. A Kaplan-Maier's product-limit estimation method was used to calculate changes in the state of survival, survival rates, and the mean survival periods. A life table was prepared using the Mantel-Haenszel method and differences in mean survival periods were examined using Peto & Peto's long-rank test. A χ^2-test with Yates' continuity correction was used on histopathology incidence data. Variables that were expressed as ranks (±) - (+++) were assessed using Wilcoxon's rank sum test. When changes in incidences exceeded 5% a dose-response relationship was tested using Mantel-Extension method.

Rats:

Weekly mean body weights and food consumption were compared using a t-test. A two-way ANOVA was used to detect differences in serial changes in body weight. Organ weight, hematology parameters, and biochemical data were converted to logarithms and examined by Gruibbs-Smirnov rejection test for the detection of abnormal values. Abnormal values were excluded from subsequent analysis. A t-test was performed on remaining data. A Kaplan- Maier's product-limit estimation method was used to calculate changes in the state of survival, survival rates, and the mean survival periods. A life table was prepared using the Mantel-Haenszel method and differences in mean survival periods were examined using Peto & Peto's long-rank test. A χ^2-test with Yates' continuity correction was used on histopathology incidence data. Variables that were expressed as ranks (±) - (+++) were assessed using Wilcoxon's rank sum test. When changes in incidences exceeded 5% a dose-response relationship was tested using Mantel-Extension method.

C. METHODS

1. Observations

Mice:

Animals were inspected once daily for general health and mortality. Moribund animals were sacrificed. In order to comply with the OPPTS 870.4300 Combined Chronic Toxicity/Carcinogenicity Health Effects Test Guidelines, the study authors should have inspected the animals twice a day for morbidity and mortality.

Rats:
Animals were inspected once daily for general health and mortality. Moribund animals were sacrificed. In order to comply with the OPPTS 870.4300 Combined Chronic Toxicity/Carcinogenicity Health Effects Test Guidelines, the study authors should have inspected the animals twice a day for morbidity and mortality.

2. Body weight

Mice:

Body weights were measured on a weekly bases for the first year and every 2 weeks, thereafter, but a significant portion of female body weight data are missing from the report. Body weights were also obtained after sacrifice with ether which was preceded by an overnight fast .

Rats:

Body weights were measured on a weekly bases for the first year and every 2 weeks, thereafter, but several weeks of the male body weight data are missing from the report. Body weights were also obtained after sacrifice with ether which was preceded by an overnight fast .

3. Food consumption, water consumption, and compound intake

Mice:

Food consumption was measured once a week on a per cage bases and mean daily diet consumption was calculated as g food/day. Water consumption was not mentioned nor reported. Food efficiency was not provided. Compound intake (g/kg bw/day) was calculated from the mean body weight, mean food consumption, and the nominal concentrations of Zeomic in the diet as no analyses were performed to test the actual levels of Zeomic in the diet.

Rats:

Food consumption was measured once a week on a per cage bases and mean daily diet consumption was calculated as g food/day. Water consumption was not mentioned not reported. Food efficiency was not provided. Compound intake (g/kg bw/day) was calculated from the mean body weight, mean food consumption, and the nominal concentrations of Zeomic in the diet as no analyses were performed to test the actual levels of Zeomic in the diet.

4. Opthalmoscopic examination

Mice:

No ophthalmoscopic examinations were conducted, as recommended per OPPTS combined chronic toxicity/carcinogenicity Health Effects Test Guidelines 870.4300. The study authors did note corneal clouding under general conditions.

Rats:

No ophthalmoscopic examinations were conducted, as recommended per OPPTS combined chronic toxicity/carcinogenicity Health Effects Test Guidelines 870.4300. The study authors did note corneal opacity under general conditions.

5. Clinical Laboratory Tests

Mice:

Blood samples obtained prior to autopsy from the subclavian venous plexus in the groin of the anterior limb were used for hematology. The first report states that 1 ml of the first sample is mixed with EDTA-2Na for hematology parameters. The second report states that EDTA-2K was added to about 1 ml of the initial blood. Animals were fasted overnight prior to blood drawing.

Rats:

Blood samples obtained prior to autopsy from the subclavian venous plexus in the groin of the anterior limb were used for hematology and clinical chemistry. The first report states that 1 ml of the first sample is mixed with EDTA-2Na for hematology parameters. The second report states that EDTA-2K was added to about 1 ml of the initial blood. An additional 7-8 ml was collected for clinical chemistry. Plasma for testing blood glucose was obtained by adding EDTA-2Na and NaF to 1 ml of blood. Serum was obtained from the remaining blood. Animals were fasted overnight prior to blood drawing.

a. Hematology

Mice:

The CHECKED (X) parameters were measured using an automatic hemocytometer (Coulter):

X	Red Blood Cell Count*	X	white blood cell count*
X	hemoglobin (HGB)*		differential leukocyte count*
X	hematocrit (HCT)*	X	platelet count*
X	mean corpuscular volume (MCV)*		Blood clotting measurement
X	mean corpuscular hemoglobin (MCH)*		(Activated partial thromboplastin time)
X	mean corpuscular hemoglobin		(Clotting time)
	concentration (MCHC)*		(Prothrombin time)

*Recommended for combined chronic toxicity/carcinogenicity studies based on OPPTS Health Effects Test Guidelines 870.4300.

Rats:

The CHECKED (X) parameters were measured using an automatic hemocytometer (Coulter):

X	Red Blood Cell Count*	X	white blood cell count*
X	hemoglobin (HGB)*		differential leukocyte count*
X	hematocrit (HCT)*	X	platelet count*
X	mean corpuscular volume (MCV)*		Blood clotting measurement
X	mean corpuscular hemoglobin (MCH)*		
X	mean corpuscular hemoglobin		
	concentration (MCHC)*		

*Recommended for combined chronic toxicity/carcinogenicity studies based on OPPTS Health Effects Test Guidelines 870.4300.

b. Clinical Chemistry

Mice:

No clinical chemistry was performed, as recommended per OPPTS combined chronic toxicity/carcinogenicity Health Effects Test Guidelines 870.4300.

Rats:

The CHECKED (X) parameters X were examined:

	ELECTROLYTES		OTHER
	potasium*	X	albumin (ALB)*
	sodium*	X	blood creatinine*
X	calcium	X	blood urea nitrogen (BUN)*
X	phosphate (inorganic)	X	total cholesterol (T-Cho)*
		X	high density lipoprotein (HDL)
	ENZYMES	X	low density lipid protein (LDL)
			globulins
X	alkaline phosphatase (ALP)*	X	glucose*
	cholinesterase	X	total bilirubin (TBL)
	creatine phosphokinase	X	total serum protein (TP)*
X	lactic acid dehydrogenase (LDH)	X	triglycerides (TG)
X	serum alanine aminotrasferase (ALT)*		serum protein electrophores
X	serum aspartate aminotransferase (AST)*	X	uric acid concentration (UA)
	gamma glutamyl transferase (GGT)		
X	amylase activity (AMS)		

* Recommended for combined chronic toxicity/carcinogenicity studies based on OPPTS Health Effects Test Guidelines 870.4300. These guidelines recommend more than 2 hepatic enzymes be measured. In addition to the enzymes listed in the table, the guidelines suggest sorbitol dehydrogenase.

6. Urinalysis

Mice:

No urinalyses were conducted as recommended per OPPTS combined chronic toxicity/carcinogenicity Health Effects Test Guidelines 870.4300.

X		X	
	appearance*		glucose*
	volume*		ketones
	specific gravity/osmolatity*		bilirubin
	pH*		blood*
	sediment (microscopic)		nitrate
	protein*		urobilinogen

* Recommended for combined chronic toxicity/carcinogenicity studies based on OPPTS Health Effects Test Guidelines 870.4300. The guidelines recommend that these parameters are measured at the end of the first year.

Rats:

No urinalyses were conducted, as recommended per OPPTS combined chronic toxicity/carcinogenicity Health Effects Test Guidelines 870.4300.

X	appearance*	X	glucose*
	volume*		ketones
	specific gravity/osmolatity*		bilirubin
	pH*		blood*
	sediment (microscopic)		nitrate
	protein*		urobilinogen

* Recommended for combined chronic toxicity/carcinogenicity studies based on OPPTS Health Effects Test Guidelines 870.4300. The guidelines recommend that these parameters are measured at the end of the first year.

7. Sacrifice and Pathology

Mice:

All animals scheduled for autopsy were fasted overnight prior to sacrifice. All animals were subject to gross pathology, including those dead or sacrificed due to morbidity. The CHECKED (X) tissues were collected for histological evaluation, and the DOUBLE CHECKED (XX) tissues indicate that these organs were also weighed. All CHECKED and DOUBLE CHECKED tissues were examined in all treatment groups. Tissues were fixed in 10% formalin. Sections of unspecified thinkness were stained in an unspecified manner for observation with a light microscope.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGICAL
X	salivary glands*		aorta*	XX	brain (multiple
X	esophagus*	XX	heart*+		sections)*+
X	stomach*		bone marrow*		pituitary*
X	duodenum*	X	lymph nodes*		peripheral nerve (sciatic
X	jejunum*	X[1]	spleen*+		or tibial)*
X	ileum*	XX	thymus		spinal cord (3 levels)*
X	cecum*			X	eyes (retina, optic
	colon*		UROGENITAL		nerve)*
	rectum*				
XX	pancreas*	XX	kidneys*+		GLANDULAR
	gallbladder*	X	urinary bladder*	XX	
XX	liver*+	X	prostate*		adrenal gland(s)*+
		X	testes*+	X	parathyroid*
	RESPIRATORY		epididymides*+		thyroid*
		X	seminal vesicles*		
X	trachea*	X	uterus*+		OTHER
X	lungs*	X	ovaries*+		
	pharynx*	X	female mammary gland*		all gross lesions and
	larynx*	X	vagina	X	masses*
	nose*			X	skin*
				X	bone (right femoral)
					muscle (right femoral)

* Recommended for combined chronic toxicity/carcinogenicity studies based on OPPTS Health Effects Test Guidelines 870.4300. + Organ weight required in combined chronic toxicity/carcinogenicity studies based on OPPTS Health Effects Test Guidelines 870.4300. The study authors just specify intestine, not specific sections of the intestine. The OPPTS Health Effects Test Guidelines recommends that lymph nodes near the area of entry be tested. The study authors tested the lymph nodes of the submandibular area. [1] The study authors do not list the spleen under histology tissues, but it is listed as an organ weight. The study authors did not provide a list of organs weighed, these are a reflection of those presented in the Results table.

Rats:

All animals scheduled for autopsy were fasted overnight prior to sacrifice. All animals were subject to gross pathology, including those dead or sacrificed due to morbidity. The CHECKED (X) tissues were collected for histological evaluation, and the DOUBLE CHECKED (XX) tissues indicate that these organs were also weighed. All CHECKED and DOUBLE CHECKED tissues were examined in all treatment groups. Tissues were fixed in 10% formalin. Sections of unspecified thinkness were stained in an unspecified manner for observation with a light microscope.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGICAL
X	salivary glands*		aorta*	XX	brain (multiple sections)*+
X	esophagus*	XX	heart*+		pituitary*
X	stomach*		bone marrow*		peripheral nerve (sciatic or tibial)*
X	duodenum*	X	lymph nodes*		spinal cord (3 levels)*
X	jejunum*	X[1]	spleen*+	X	eyes (retina, optic nerve)*
X	ileum*	XX	thymus		
X	cecum*		UROGENITAL		GLANDULAR
	colon*			XX	
	rectum*				adrenal gland(s)*+
XX	pancreas*	XX	kidneys*+	X	parathyroid*
	gallbladder*	X	urinary bladder*		thyroid*
XX	liver*+	X	prostate*		
		X	testes*+		OTHER
	RESPIRATORY		epididymides*+	X	
		X	seminal vesicles*		all gross lesions and masses*
X	trachea*	X	uterus*+	X	skin*
X	lungs*	X	ovaries*+	X	bone (right femoral) muscle (right femoral)
	pharynx*	X	female mammary gland*		
	larynx*	X	vagina		
	nose*				

* Recommended for combined chronic toxicity/carcinogenicity studies based on OPPTS Health Effects Test Guidelines 870.4300. + Organ weight required in combined chronic toxicity/carcinogenicity studies based on OPPTS Health Effects Test Guidelines 870.4300. The study authors just specify intestine, not specific sections of the intestine. The OPPTS Health Effects Test Guidelines recommends that lymph nodes near the area of entry be tested. The study authors tested the lymph nodes of the submandibular area. [1] The study authors do not list the spleen under histology tissues, but it is listed as an organ weight. The study authors did not provide a list of organs weighed, these are a reflection of those presented in the Results table.

II. RESULTS

A. Observations

1. Toxicity

Mice:

A dark pigmentation of the skin was observed in mice treated with 0.9%. The study authors note that there are treatment related differences in body weight gain in male mice. Although

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there were sporadic changes in organ weights, the authors did not attribute any changes to the treatment. There were fluctuations in hematology parameters. Those that the study authors attributed to treatment were decreases in RBC, hemoglobin, hematocrit, MCV and MCH in male mice treated with 0.9%. Although all groups for both sexes exhibited a dose response in pigmentation of several organs, the authors attribute this to the deposition of silver and not to a toxic effect. The study authors did not note any increases in tumor development. The study author did not determine a NOAEL for the mice. Our reviewer found inconsistences in the data presented and the study authors conclusions. Each is reported in their respective sections that follow.

Rats:

A dark pigmentation of the skin was observed in rats treated with 0.1 or 0.3%. The study authors note that there are treatment related differences in body weight gain in female rats. Although there were sporadic changes in organ weights, the authors did not attribute any changes to the treatment. There were fluctuations in the clinical chemistry and hematology parameters. Those that the study authors attributed to treatment were ALT, ALB and TP increase in the male and female rats and LDH decreases in male and female rats. The study author found a significant dose-response relationship for leukemia in female rats. The study authors did not attribute it to treatment since the individual treatments were not statistically different from the controls and the values for all groups were lower than historical control incidence. Although all groups for both sexes exhibited a dose response in pigmentation of several organs, the authors attribute this to the deposition of silver and not to a toxic effect. The study author determined the NOAEL to be 0.011 g/kg/day based on female rat data from the 0.03% group. Our reviewer found inconsistences in the data presented and the study authors conclusions. Each is reported in their respective sections that follow.

2. Mortality

Mice:

Compared to control male mice, overall survival in treated male mice was unaffected at study termination by treatment with the test chemical. In female mice, mortality was not negatively affected by treatment, and survival at the high dose appeared greater than in control female mice. The survival did not fall below 50% at 15 months for male and female mice in any group. All groups also had greater that 25% surviving at 18 months. Therefore, the survival was within that recommended in the OPPTS Health Effects Test Guidelines 870.4300 for combined chronic toxicity/carcinogenicity studies.

Table 3. Mortality (number dead/total) in Male and Female Mice

Time Interval	Dose Group (% in diet)			
	0	0.1	0.3	0.9
Males				
15 months	0/50	0/50	2/50	1/50
18 months	4/50	5/50	9/50	3/50
24 months	13/50	13/50	19/50	14/50
Females				
15 months	1/50	0/50	0/50	1/50
18 months	3/50	2/50	3/50	1/50
24 months	12/50	10/50	8/50	9/50

Results were obtained from Tables 5-1 (males) and 5-2 (females) in the study report.

Rats:

The mortality was similar in all treatment groups and their respective controls. The survival did not fall below 50% at 18 months for the rats in any group. All groups also had greater that 25% surviving at 24 months. See Table 4 for details. Therefore, the survival was within that recommended in the OPPTS Health Effects Test Guidelines 870.4300 for combined chronic toxicity/carcinogenicity studies.

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Table 4. Mortality (number dead/total) in Male and Female Rats

Time Interval	Dose Group (% in diet)				
	0	0.01	0.03	0.1	0.3
Males					
15 months	0/50	1/50	0/50	0/50	1/50
18 months	1/50	6/50	5/50	6/50	4/50
24 months	26/50	23/50	18/50	24/50	24/50
Females					
15 months	1/50	0/50	1/50	0/50	1/50
18 months	3/50	2/50	2/50	1/50	1/50
24 months	17/50	15/50	17/50	13/50	18/50

Results were obtained from Tables 15-1 (males) and 15-2 (females) in the study report.

B. Body weight and weight gain

Mice:

The study authors noted a significant depression in body weight gain in male mice treated with 0.3 and 0.9% Zeomic. The 0.3% group body weight was depressed from week 18 to week 65 and the 0.9% group was depressed from week 22 to week 85. There was no suppression in male mice treated with 0.1% or in any treated female mice.

The study authors appear to use body weight and body weight gain interchangeably. Table 6-1 in the study report demonstrates significant differences in body weight at the times mentioned by the study authors, but according to the data calculated by our reviewer and presented here in Table 5 the body weight gain in the male is affected in the first 18 weeks for all groups and to week 65 in the 0.9% group. The study authors also fail to mention the significant decrease in body weight in male mice observed in the 0.1% group from 19-79 weeks. The largest decrease in total body weight gain was observed in the 0.1% group (see Tables 5 and 6). Since the study authors failed to provide all the weekly data for the female mice our reviewer was not able to make the same comparison in the female mice. The

missing data were not noted before the report was submitted as indicated by the consecutive page numbers of the study report. The data that are provided demonstrate various points of statistical increase in the body weight of females treated with 0.1% and very little changes in the other two groups. Since the study authors present body weight in two different places (i.e., the mean weekly weights of the groups and the initial and final weights of the animals for sacrifice at the different time points), two tables were made by our reviewer. The data for the male mice at sacrifice presented here in Table 6 adhere more to the study authors claim that body weight gain was suppressed during the middle time points. By 24 months the totals demonstrated the same findings (Table 5 and 6). The results calculated by our reviewer and presented in Table 6 on the females agrees with the authors findings that there were minimal changes in body weight gain of the female mice, but the 0.9% caused a 7% depression while 0.1 and 0.3% caused an increase in body weight gain. In terms of the maximum tolerated dose (MTD), the high dose in male mice caused an increase in food consumption for the whole study (10%), but a decrease in the absolute body weight from 18-101 weeks (4%) and a decrease in body weight gain (6%) for the duration of the study (Table 7). This may be an indication of a MTD, but cannot be corroborated by data in the females due to missing data for a significant portion of the study (weeks 0-17 and 40-71). Although the MTD may not have been obtained, the study authors did achieve the limit dose of 1000 mg/kg bw/day (Table 11) that is recommended in the OPPTS combined chronic toxicity/carcinogenicity Heath Effects Test Guidelines 870.4300.

Table 5. Body Weight Gain (g) in Male and Female Mice at Selected Intervals and Over the Study

Time Interval (weeks)	Dose Group (% in diet)			
	0	0.1	0.3	0.9
Males				
0-18	18.6	17.4 (↓6%)	17.1 (↓8%)	17.8 (↓4%)
18-65	9.3	9.3 (0%)	9.6 (↑3%)	7.8 (↓18%)
65-85	-4	-4.4 (↓10%)	-2.6 (↑35%)	-3.5 (↑12%)
85-101	-4.3	-4.7 (↓9%)	-3.3 (↑24%)	-3.6 (↑16%)
total	19.6	17.6 (↓10%)	20.8 (↑6%)	18.5 (↓6%)
Females				
0-18	The study authors failed to report data for			
18-65	weeks 0 to 17			
65-85	and weeks 40 to 70			

Time Interval (weeks)	Dose Group (% in diet)			
	0	0.1	0.3	0.9
85-103	0.4	1.4 (↑250%)	2.1 (↑425%)	1.2 (↑200%)
total	cannot compute due to missing data			

Results were calculated using the data provided by the study authors in Table 6-1 in the study report (pg 131-136 of the report).

Table 6. Body Weight Gain (g) in Male and Female Mice at Interim and Terminal Sacrifice

Time Interval (months)	Dose Group (% in diet)			
	0	0.1	0.3	0.9
Males				
3 (n=5/group)	16.5	17.5 (↑6%)	12.8 (↓22%)	17.2 (↑4%)
6 (n=10/group)	22.7	22.2 (↓2%)	20.4 (↓10%)	21.3 (↓6%)
12 (n=10/group)	27.9	27.3 (↓2%)	25.7 (↓8%)	25.7 (↓8%)
24 (n=survivors)	19.9	18 (↓10%)	20.8 (↑4.5%)	18.4 (↓8%)
Females				
3 (n=5/group)	8.7	11.9 (↑37%)	8.7 (0%)	9.8 (↑13%)
6 (n=10/group)	9.8	12.1 (↑23%)	10.3 (↑5%)	11.5 (↑17%)
12 (n=10/group)	25.2	28.5 (↑13%)	23.9 (↓5%)	26 (↑3%)
24 (n=survivors)	33.1	35 (↑6%)	34.3 (↑4%)	30.8 (↓7%)

Results were calculate from the data presented by the study authors in Tables 8-1 (males) and 8-2 (females) of the study report (pg. 137-138). Results were from fasted animals.

Table 7. Average Absolute Body Weight (g) and Average Food Consumption (g/day) in Male and Female Mice at Selected Intervals and Over the Study

Time Interval (weeks)	Dose Group (% in diet)			
	0	0.1	0.3	0.9
Males				

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Time Interval (weeks)	Dose Group (% in diet)							
	0		0.1		0.3		0.9	
	average body weight	Ave Food consum	average body weight	Ave Food consum	average body weight	Ave Food consum	average body weight	Ave Food consum
0-18	32.7	4.7	32.4 ↓1%	4.9 ↑3%	32.5 ↓1%	5.1 ↑8.5%	32.9 ↑1%	5.3 ↑13%
18-65	47.6	4.4	46.2 ↓3%	4.7 ↑6%	45.1 ↓5%	4.7 ↑6%	45.6 ↓4%	4.7 ↑6%
65-85	47.6	3.7	46 ↓3%	3.6 ↓3%	47.1 ↓1%	3.9 ↑5%	45.6 ↓4%	4 ↑8%
85-101	43	3.2	41 ↓5%	3.2 0%	43.7 ↑2%	3.6 ↑12.5%	41.8 ↓3%	3.6 ↑12.5%
Females								
	average body weight	Ave Food consum	average body weight	Ave Food consum	average body weight	Ave Food consum	average body weight	Ave Food consum
0-18	The study authors failed to provide data for this time period.							
18-39	33	4	34.7 ↑5%	4.1 ↑3%	33.5 ↑2%	3.9 ↓3%	33.9 ↑3%	3.9 ↓3%
40-71	The study authors failed to provide data for this time period.							
71-103	50.3	3.7	52 ↑3.5%	3.7 0%	50.4 ↑0.2%	3.9 ↑4%	48.2 ↓4%	3.6 ↓2%

Results were calculated from Table 6-1 in the study report.

Rats:

The study authors note that male rats had a suppression in body weight as follows: week 4 to week 53 in the 0.01% group, week 4 to week 33 in the 0.03% group, week 2 to week 49 in the 0.1% group, and week 3 to week 32 in the 0.3% group. These depressions were not maintained through the study duration and at termination there were no significant differences between treatment and control groups in the male rat. The decrease in female body weight was not consistent during weeks 35-102 and was not evident at termination (weeks 104 to 106). Female rats had a significant reduction in body weight gain in the 0.3%

group only from week 35 to week 102. Body weight data for the females showed statistically significant decreases at the high dose that were not consistent during the study and were less than 10% (Table 10). These data do not indicate that a MTD was achieved based on body weight. Missing body weight data for the males (weeks 68-106) makes interpretation of the MTD difficult, since statistically significant effects on male body weight were not consistent after 32 weeks.

Our reviewer agrees that there is an early depression in weight gain in the male rats that is not maintained to week 68 in the males. Our reviewer calculated body weight gains over various intervals and the total over the study duration using the weekly data provided by the study authors. The results are tabulated in Table 8. Since the study authors failed to provide the weekly data from 69 to the study end in the male rat, our reviewer was not able to make the determination through the end of the study using the weekly data. This missing data were not noted before the report was submitted as indicated by the consecutive page numbers in the study report. Since the study authors present body weight in two different places (i.e., the mean weekly weights of the groups and the initial and final weights of the animals for sacrifice at the different time points), two tables were made by our reviewer. The data for the male rats at sacrifice presented in Table 9 suggests that the male rats treated with 0.3% had a slight depression in weight gain by the study end. Our reviewer also agrees that in female rats the 0.3% group was the only group that had a consistent depression in body weight gain, but our reviewer notes that at the end of the study all groups had slightly depressed body weight gains (Table 9). Female rats had a consistent depression in body weight gain in the 0.3% group, while the other 3 groups had an initial increase in body weight gain that was not evident at the terminal sacrifice (Table 9). Overall, the effects on the body weight of rats do not indicate that a MTD was achieved. The study authors also did not approach the 1000 mg/kg bw/day that is considered the upper limit in OPPTS combined chronic toxicity/carcinogenicity Health Effects Test Guidelines 870.4300.

Table 8. Body Weight Gain (g) in Male and Female Rats at Selected Intervals and Over the Study

Time Interval (weeks)	Dose Group (% in diet)				
	0	0.01	0.03	0.1	0.3
Males					
0-12	242.5	227.9 (↓4%)	236.6 (↓2%)	223.7 (↓8%)	234.7 (↓3%)
12-26	73.6	65.8 (↓11%)	68.6 (↓7%)	67.9 (↓8%)	70.4 (↓4%)
26-52	52.8	61.6 (↑17%)	70.9 (↑34%)	73 (↑38%)	65.4 (↑24%)
52-60	0.7	7.9 (↑1000%)	3.7 (↑428%)	7.1 (↑900%)	7.5 (↑1000%)
60-68	5.7	11.5 (↑100%)	-7.9 (↓230%)	-1.5 (↓126%)	2 (↓65%)

Time Interval (weeks)	Dose Group (% in diet)				
	0	0.01	0.03	0.1	0.3
68-106	The study authors do not provide the data for this time period.				
total (to 68 weeks)	375.3	374.7 (↓0.2%)	371.9 (↓1%)	370.2 (↓1.4%)	380 (↑1.3%)
Females					
0-12	117.4	113.1 (↓4%)	117.4 (0%)	117.4 (0%)	116.9 (↓0.5%)
12-26	36.1	36.8 (↑2%)	36.9 (↑2%)	35.5 (↓2%)	35.1 (↓3%)
26-52	66.9	64 (↓4%)	65.7 (↓2%)	63.8 (↓5%)	58.8 (↓12%)
52-60	20.1	29.5 (↑47%)	25.5 (↑27%)	26.4 (↑31%)	24.9 (↑24%)
60-78	31.9	30.9 (↓3%)	31.7 (↓1%)	28.4 (↓11%)	28.4 (↓11%)
78-106	8.6	-14.3 (↓266%)	-4.9 (↓157%)	0.4 (↓95%)	-3.2 (↓137%)
total	281	260 (↓7%)	272.3 (↓3%)	271.9 (↓3%)	260.9 (↓7%)

Results were calculated from the data provided by the study authors in Tables 16-1 (males) and 16-2 (females) in the study report.

Table 9. Body Weight Gain (g) in Male and Female Rats at Interim and Terminal Sacrifice

Time Interval (months)	Dose Groups (% in diet)				
	0	0.01	0.03	0.1	0.3
Males					
6	298.6	281.2 (↓6%)	294.5 (↓1%)	280.2 (↓6%)	288.7 (↓3%)
12	345.6	335.8 (↓3%)	355.3 (↑3%)	352.3 (↑2%)	347.5 (↑0.5%)
24	349.1	349.8 (↑0.2%)	353.4 (↑1%)	339 (↓3%)	333.5 (↓4.5%)
Females					
6	138.2	141.8 (↑3%)	139.2 (↑1%)	139 (↑0.5%)	137.6 (↓0.5%)
12	215.4	197.9 (↓8%)	205 (↓5%)	205.1 (↓5%)	195 (↓9.5%)
24	283	268.4 (↓5%)	282.6 (↓0.2%)	278.6 (↓2%)	262.1 (↓7%)

Results were calculated from the data provided by the study authors in Tables 19-1 (males) and 19-2 (females) in the study report.

Table 10. Average Absolute Body Weight (g) and Food Consumption (g/day) in Male and Female Rats at Selected Intervals and Over the Study

Time Interval (weeks)	Dose Group (% in diet)									
	0		0.01		0.03		0.1		0.3	
Males										
	average body weight	Ave Food consum	average body weight	Ave Food consum	average body weight	Ave Food consum	average body weight	Ave Food consum	average body weight	Ave Food consum
0-12	245.3	14.1	236.7 ↓4%	13.7 ↓3%	240.7 ↓2%	14.1 0%	236.5 ↓4%	13.3 ↓6%	242.7 ↓1%	14 ↓1%
12-26	380.1	14.6	362 ↓5%	13.7 ↓6%	369.8 ↓3%	14.2 ↓3%	356.4 ↓4%	13.6 ↓7%	368.7 ↓3%	14 ↓4%
26-52	444.1	15.2	425.5 ↓4%	14.7 ↓3%	441.7 ↓0.5%	15.5 ↑2%	429.5 ↓3%	15.3 ↑0.7%	439.5 ↓1%	15.7 ↑3%
52-60	464.4	15.5	455.5 ↓2%	15.8 ↑2%	469.7 ↑1%	16 ↑3%	462.3 ↓0.5%	16.3 ↑5%	468 ↑1%	16.2 ↑4.5%
60-68	465	14.8	462.1 ↓1%	14.9 ↑0.7%	466.4 ↑0.3%	15 ↑1%	462.8 ↓0.5%	14.7 ↓0.7%	471 ↑1%	15.1 ↑2%
68-106	The study authors did not provide data for this time period.									
Females										

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Time Interval (weeks)	Dose Group (% in diet)									
	0		0.01		0.03		0.1		0.3	
	average body weight	Ave Food consum	average body weight	Ave Food consum	average body weight	Ave Food consum	average body weight	Ave Food consum	average body weight	Ave Food consum
0-12	160.2	9.8	157.9 ↓1.5%	9.6 ↓2%	160 ↓0.2%	10 ↑2%	160.4 ↑0.2%	9.8 0%	160 ↓0.2%	9.85 ↑0.05%
12-26	222.4	9.5	218.3 ↓2%	9.2 ↓3%	222.3 0%	9.55 ↑0.5%	221.6 ↓0.4%	9.5 0%	221 ↓0.6%	9.4 ↓1%
26-52	273	10.7	267.5 ↓2%	10.4 ↓3%	272.7 ↓0.2%	10.6 ↓1%	281 ↑3%	10.7 0%	265.8 ↓3%	10.5 ↓1.5%
52-60	314.7	11.6	314.2 ↓0.2%	11.9 ↑2.5%	316.7 ↑0.6%	11.8 ↑2%	318.2 ↑1%	11.7 ↑1%	308.5 ↓2%	11.7 ↑1%
60-78	345.2	11.4	345.2 0%	11.5 ↑1%	349 ↑1%	11.6 ↑2%	347 ↑0.5%	11.2 ↓2%	334.7 ↓3%	11.3 ↓1%
78-106	364.7	11.6	360 ↓1%	11.3 ↓2%	365.7 ↑0.3%	11.6 0%	366.3 ↑0.4%	11.6 0%	349 ↓4%	11.6 0%

C. Food consumption, water consumption and compound intake

1. Food consumption

Mice:

Overall, the study authors noted that the food consumption was increased in the males and decreased in the females. The increases were observed in the males from week 18 to week 51 weeks in the 0.1% group and from week 18 to the study termination in the 0.3% and 0.9% groups. The decreases in the females were observed from week 3 to week 30 in the 0.3% group and from week 15 to week 93 weeks in the 0.9% group. Our reviewer agrees that there was a consistent increase in the food consumption in the males during the middle period of the study. Since the study authors failed to present weeks 0 to 17 and 40 to 70 for female mice in the study report, our reviewer could only assess the data present . The missing data were not noted before the report was submitted as indicated by the consecutive page numbers in the study report. The data that were present did demonstrate a decrease in consumption for the females treated with 0.3 and 0.9%. Our reviewer has compiled the food consumption data to the extent possible and have presented it here in Table 7 (above).

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Rats:

The study authors report that there were weekly decreases in food consumption in all treated groups, but these periods of low food intake were followed by a period of increased food consumption. Any weekly changes were not persistent over time. Our reviewer agrees that there were periods of decreased food intakes followed by increased food intakes, but it appears in the males that there were more periods of decreased food intake. Our reviewer has compiled the food consumption data to the extent possible and have presented it here in Table 10 (above).

2. Water consumption

Mice:

The study authors do not report the amount of water consumed.

Rats:

The study authors do not report the amount of water consumed.

3. Compound intake

Mice:

The study authors report a provide drug intake data (g/kg bw/day) and cumulative drug intake (g/mouse) for weekly and or biweekly time points. The average intake for the 101 weeks in males is presented in Table 11. Females could not be evaluated due to missing weeks 0-17 and 40-70. The drug intake is the highest at the beginning of the study and decreases as the study progresses. These numbers are based on the nominal percentages in the diet. Since there is no evidence in the report that the study authors or the providers of the diet (i.e., Oriental Yeast Co.) test the levels in the diet, these numbers are based on assumed heterogeneity in the diet and the correct percentage present in the diet (i.e., nominal not analytical concentrations).

Table 11. Average Drug Intake (mg/kg bw/day) in Male Mice

	Dose (% in diet)		
	0.1	0.3	0.9
Average drug intake mg/kg bw/day	106	340	1015

Results were obtained from Table 6-1 in the study report.

Rats:

The study authors provide both drug intake (g/kg bw/day) and cumulative drug intake at weekly and/or biweekly intervals. The average intake for the first 68 weeks in males and 106 weeks in females is presented in Table 12. The drug intake is the highest at the beginning of the study and decreases as the study progresses. These numbers are based on the target percentages in the diet. Since there is no evidence in the report that the study authors or the providers of the diet (i.e., Oriental Yeast Co.) test the levels in the diet, these numbers are based on assumed heterogeneity in the diet and the correct percentage present in the diet (i.e., nominal, not analytical, concentrations).

Table 12. Average Drug Intake (mg/kg bw/day) in Male (0-68 weeks) and Female (0-106 weeks) Rats

	Dose (% in diet)			
	0.01	0.03	0.1	0.3
Males				
Average drug intake mg/kg bw/day	4.1	12.2	39.5	121
Females				
Average drug intake mg/kg bw/day	4.1	12.5	41.1	125

Results were obtained from Table 16-1 (males) and 16-2 (females) in the study report.

D. Opthalmoscopic examination

Mice:

No ophthalmoscopic examinations were conducted, as recommended per OPPTS combined chronic toxicity/carcinogenicity Health Effects Test Guidelines 870.4300. The study authors did note cloudy corneas in the general conditions.

Rats:

No ophthalmoscopic examinations were conducted, as recommended per OPPTS combined chronic toxicity/carcinogenicity Health Effects Test Guidelines 870.4300. The study authors did note cornea opacity in the general conditions.

E. Blood work

1. Hematology

Mice

The study authors reported that the high dose (0.9%) group had reduced RBC, hemoglobin, hematocrit, MCV and MCH values in comparison to the controls for both males and females. The study authors also note decreases for these parameters in the 0.3% dose group at 6 and 12 months but not at 24 months in the males and at 6 and 24 months but not at 12 months in the females. Platelet counts were decreased at various time points during the study in both sexes treated with 0.3% or 0.9%, but the depression wasn't maintained till the study duration. Our reviewer agrees that there was a consistent depression in RBC, hemoglobin, hematocrit, MCV, and MCH values for the 0.9% dose group in both males and females as shown in Table 13 and 14. The 0.3% group had decreases, but they could not be unequivocally associated with treatment induced anemia since trends were different in the males and females and they were transient. The study authors did not report a NOAEL based on hematology. However it appears that the NOAEL is 0.1% and the LOAEL is 0.3%, based on decreased RBC, hemoglobin, hematocrit, MCV, and MHC at 24 months.

Table 13. Hematology Parameters where Effects were Noted in Male Mice at all Time Intervals

Hematology parameter	Time Interval															
	3 month				6 months				12 months				24 months			
	Males															
	Dose (% in diet)				Dose (% in diet)				Dose (% in diet)				Dose (% in diet)			
	0	0.1	0.3	0.9	0	0.1	0.3	0.9	0	0.1	0.3	0.9	0	0.1	0.3	0.9
RBC	862.5	878.8	874.6	733.5 **	942.3	915	891.6 *	782 **	952.8	933.8	924.7	797.6 **	808.3	817.9	781.8	683.1 **
hemoglobin	14.1	14.32	14.12	12.13 **	14.95	14.46	14.08 **	12.08 **	15.5	15.03	14.72 **	12.09 **	12.78	12.76	12.2	10.45 **
hematocrit	41.68	42.4	41.28	33.45 **	45.23	43.31 *	42.11 **	34.77 **	48.24	46.83	46.09 *	36.71 **	36.59	36.26	34.92	30.12 **
MCV	48.33	48.28	47.20 **	45.63 **	48.03	47.34 *	47.21 *	44.48 **	50.51	50.15	49.82	46.55 **	45.33	44.57 **	44.84	43.96 **
MHC	16.35	16.3	16.14	16.53	15.88	15.81	15.79	15.41 **	16.23	16.10	15.94 **	15.15 **	15.79	15.73	15.67	15.53 **

Data are the means presented by the study authors in Table 7. * denotes $p<0.05$ and ** $p<0.01$ as indicated by the study author.

Table 14. Hematology Parameters where Effects were Noted in Female Mice at all Time Intervals

Hematology parameter	Time Interval															
	3 months				6 months				12 months				24 months			
	Females															
	Dose (% in diet)				Dose (% in diet)				Dose (% in diet)				Dose (% in diet)			
	0	0.1	0.3	0.9	0	0.1	0.3	0.9	0	0.1	0.3	0.9	0	0.1	0.3	0.9
RBC	550	844.8	874	813.6 **	900.4	901.4	878.9 *	796.3 **	887.2	890	894.6	841.4 **	791.7	797.6	760.7 *	682.2 **
hemoglobin	14.86	13.96	14.52	13.40 **	14.23	14.33	13.77 **	12.50 **	14.22	14.52 *	14.24	12.69 **	13.19	13.08	12.41 **	10.79 **
hematocrit	42.58	40.88 *	41.76	36.90 **	43.57	43.54	41.73 **	35.49 **	43.47	44.82 *	43.77	38.41 **	38.68	37.15 *	35.50 **	30.25 **
MCV	48.92	48.38	47.78 *	45.34 **	48.39	48.32	47.50 *	44.56 **	49.54	50.33	48.95	45.63 **	48.44	46.82 **	46.74 **	44.33 **
MHC	16.90	16.52	16.62	16.50	15.80	15.90	15.67	15.72	16.17	16.16	15.91 *	15.10 **	16.56	16.43	16.38	15.78 **

Data are the means presented by the study authors in Table 7. * denotes $p<0.05$ and ** $p<0.01$ as indicated by the study author.

Rats

The study authors note that there were statistical changes in various parameters at various times, but there were no changes that could be related to treatment. Although they did not believe changes were related to treatment, they noted significant decreases in RBC, HGB, HCT, MCH, and MCHC in the 0.3% group and decreases in HGB, HCT, MCH, and MCHC in the 0.1% group at 24 months which they claimed suggested microcytic hypochromic anemia. Our reviewer believes that there was a treatment related decrease in RBC at 24 months for both males (823.5 vs 739.0; $p<0.05$) and females (748.3 vs 683.7; $p<0.05$) treated with 0.3% (see Tables 15). Although this may be the case, males demonstrated an increase in RBC at the 6 and 12 months time point for both the 0.1% and 0.3% dose groups. The male rats treated with 0.3% had reduced WBC at 6 and 12 months. The females had slight, but not statistical reductions in WBC at 6 months in the 0.3% group. The reduction was statistically significantly different from the control by 12 months. By 24 months, the WBC count appears very high, although the study authors do not have this marked as statistically significantly different from the control in the males (8.59 control vs 44.17 treatment with 0.3%) it is significantly increased in the females (8.2 control vs 19.18 treatment with 0.3%). The study authors also do not provide the individual data for males treated with 0.3%. The missing data were not noticed prior to submission as noted by the consecutive page numbers in the study report. Therefore, this number could not be verified. Individual female values were in the range of the control values with the exception of 5 values that were high (data from Reference 8-3) but were not considered outliers. Our reviewer also felt that the females had a treatment related decrease in hemoglobin and hematocrit at 24 months. Both values seem to have dose-response relationships with statistical significance obtained in the 0.1 and 0.3% group for hemoglobin and in the 0.03, 0.1, and 0.3% groups for hematocrit. The study authors did not report a NOAEL based on hematology. However it appears that the NOAEL is 0.03%, based on decreased RBC, hemoglobin, hematocrit, MCV, and MHC and the LOAEL is 0.1%.

Table 15 . Hematology Parameters where Effects Noted in Female Rat treated for 24 months

Hematology parameter	Dose (% in diet)				
	0	0.01	0.03	0.1	0.3
RBC	748.3	735.1	748.2	754.8	683.7*
hemoglobin	15.11	14.90	14.77	14.24**	13.30**
hematocrit	41.19	41.64	40.05*	39.53*	37.11**
MHC	20.18	20.01	20.10	19.59**	19.61**
MCHC	36.31	36.59	36.80	36.08**	35.59**

Results were obtained from Table 17 in the study report. * $p<0.05$; ** $p<0.01$.

2. Clinical Chemistry

Mice:

Serum biochemistry was not performed on the mice, as recommended per OPPTS combined chronic toxicity/carcinogenicity Health Effects Test Guidelines 870.4300.

Rats:

Although the study authors report in their summary that there was an increase in ALT, ALB, and TP in both males and females treated with 0.3%, they report in their results that there is an increase in ALT and ALP, but a decrease in TP and ALB. The study authors report that the LDH activity was lower in all treatment groups than the controls. They claim that there are other various changes in parameters related to lipid metabolism and endocrine/exocrine functions of the pancreas, but none of the changes were consistent over time and did not appear to be dose dependent. They claim that there are no changes in parameters which measure renal function. Our reviewer found that there was a decrease in TP and ALB, but the decreases were not constant over the time periods and although the study authors found the values to be statistically different from the control they were minimally different from the control. Our reviewer found that there was a decrease in LDH for all time periods in both males and females, but a decrease in LDH is not associated with any toxic effects. The ALT and ALP values were statistically significantly increased from the control at 24 months for both males and females treated with 0.3% indicating a possible effect on the liver (Table 16). Our reviewer found there to be no differences in the other parameters measuring lipid content of the serum, but they varied over time and did not appear to be related to treatment. Although our reviewer did not find any effect on BUN or creatinine, there appeared to be a treatment-related decrease in uric acid levels in the males (Table 16). The study authors did not report a NOAEL based on clinical chemistry parameters. However it appears that the NOAEL is 0.1%, based on increased ALT and ALP and the LOAEL is 0.3%.

Table 16. Select Clinical Chemistry Parameters at Select Time Points in the Male and Female Rats

Clinical Chemistry Parameter	Dose (% in diet)				
	0	0.01	0.03	0.1	0.3
Males					
Uric Acid 6 months	2.00	1.59*	1.64*	1.33**	1.07**
Uric Acid 12 Months	2.07	1.82	1.90	1.28**	0.99**
Uric Acid 24 months	2.37	2.35	2.23	1.64**	1.90**
ALT 24 months	33.3	41.1 *	40.1*	44.1*	90.7**
ALP 24 months	138.4	126.7	119*	142.8	184.5*
Females					
ALT 24 months	40.9	39.6	36.1*	38.6	64.6*
ALP 24 months	79.1	82.3	76.9	85.5	109.9**

Results were obtained from Table 18-1in the study report. * $p<0.05$; ** $p<0.01$

F. Urinalysis

The study authors did not perform urinalysis, as recommended per OPPTS combined chronic toxicity/carcinogenicity Health Effects Test Guidelines 870.4300.

G. Sacrifice and Pathology

1. Organ weight

Mice:

The study authors claim that there were changes in organ weights in both the males and females, but that the changes were not considered related to treatment as they did not persist through all time points. Decreases were observed in liver weight of males treated with 0.9%

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at 3, 6, and 12 months, but not at 24 months and in the females at 12 and 24 months (Table 17). The pancreas weight was increased in the males treated with 0.9% at 12 and 24 months (Table 17). The study authors did not report a NOAEL based on organ weights. However it appears that the NOAEL is 0.1%, based on the increased weight of the pancreas and the LOAEL is 0.3%.

Table 17. Organ Weights of Select Organs (g) in Male and Female Mice at Interim and Terminal Sacrifice

select organs	Time Interval															
	3 months				6 months				12 months				24 months			
	0	0.1	0.3	0.9	0	0.1	0.3	0.9	0	0.1	0.3	0.9	0	0.1	0.3	0.9
Males																
liver	1.55	1.59	1.26 **	1.64	1.79	1.68	1.61	1.60 *	2.04	1.89	1.79 *	1.59 **	1.89	1.67	1.80	1.80
Pancreas	0.15	0.18	0.15	0.19	0.24	0.18 **	0.23	0.26	0.28	0.26	0.25	0.39 *	0.21	0.21	0.24 *	0.25 **
Females																
liver	1.07	1.27 **	1.11	1.23 *	1.14	1.14	1.05	1.10	1.61	1.51	1.43	1.31 **	1.93	2.07	1.90	1.72 *

Results were obtained from Tables 19-1 (males) and 19-2 (females) in the study report. * $p<0.05$; ** $p<0.01$.

Rats:

The study authors report decreases in several organs (i.e., thymus, liver, kidney, spleen and pancreas) in some of the treatment groups after 6 and 12 months in the males and an increase in the weights of the pancreas and adrenals at 24 months. The study authors did not relate these observations to treatment as there was no dose-response relationship in any of the organs. The study authors report that there were no changes observed in the females. Our reviewer agrees that there doesn't appear to be any treatment-related effects on organ weights in either the males or females. Therefore, the NOAEL is 0.9% and the LOAEL would be greater than 0.9%.

2. Gross pathology

Mice:

The study authors note that there was increased pigmentation in many of the organs in both sexes. They also noted a dose-response relationship to the treatment level and the level of pigmentation. The study authors summarize the findings in tables 13-1 (male mice) and 13-2 (female mice) of the study report. Our reviewer agrees that there is definitely a dose-response relation in the number of animals with pigmentation and the severity of pigmentation with the liver, kidneys, stomach, lymph nodes, and pancreas being the greatest effected. This effect is due to silver deposition and may not be toxicologically significant. There were no other gross observations that appear treatment related. Therefore, the NOAEL was 0.9% and the LOAEL would be greater than 0.9%.

Rats:

The study authors note that there was increased pigmentation in many of the organs in both sexes. They also noted a dose-response relationship to the treatment level and the level of pigmentation. The study authors summarize the findings for the female rat in table 24-2 of the study report. Although the study authors provided a dose-response relations table of pigmentation for the male rats, they did not provide a table detailing the occurrence of pigmentation with the severity of pigmentation as was provided for the female rats. The missing data were not noted prior to submission of the report as noted by the consecutive page numbering in the study report. Our reviewer agrees that there is definitely a dose-response relation in the number of animals with pigmentation and the severity of pigmentation with the liver, kidneys, and pancreas being the greatest effected in the rats. This effect is due to silver deposition and may not be toxicologically significant. There were no other gross observations that appear treatment related. Therefore, the NOAEL was 0.9% and the LOAEL would be greater than 0.9%.

3. Microscopic pathology

a) Non-neoplastic

Mice:

The study authors noted many non-neoplastic changes in all mice sacrificed at 24 months including the controls. There were a few sporadic changes that occurred at 6 and 12 months. The greatest number of changes observed were cardiac thrombosis, pulmonary edema, calcification of the brain, renal cysts, siliconuria, enlargement of the Langerhans' islands, fatty liver, and ovaries cysts. Dose-response relationships were found for the enlargement of Langerhans' islands in the males and renal cysts in the males and females. The study authors provide a dose-response relationship table in the study report (Table 12 of the study report). Although the study authors did not find a dose-response trend in ovarian cysts in the females,

there was a statistically significant increase in ovary cysts for all treated groups in comparison with the control. In the males there was a significant increase in non-neoplastic changes in males treated with 0.3 and 0.9% as can be seen in Table 18. Although all treated females had a greater incidence of non-neoplastic changes, only those treated with 0.1% had a statistically significant increase. Our reviewer compiled the total number of mice bearing non-neoplastic changes into Table 19. Because the study authors provided only data combining the animals that died or were sacrificed with the animals that survived the 24 months, a more detailed analysis of these results is needed to evaluate these effects and establish toxicological significance.

Table 18. Significant Non-neoplastic Changes in Male and Female Mice at 24 Months

Non-neoplastic parameter	Dose (% in diet)			
	0	0.1	0.3	0.9
	Males			
enlargement of Langerhans' island[1]	3/49	7/48	13/49*	11/50*
renal cysts[2]	0/49	0/48	0/49	4/50
	Females			
renal cysts[1]	0/49	0/49	1/50	3/49
ovarian cysts	6/49	22/49**	19/50**	16/49*

Data were derived from Tables 11-2 and 11-3 of the study report. * $p<0.05$ and ** $p<0.01$ as indicated in the study report.

[1] The study author notes that this observation had a dose-response relationship that was statistically significant with a $p<0.05$. [2] The study author notes that this observation had a dose-response relationship that was statistically significant with a $p<0.01$.

Table 19. Total Number and Percent of Mice with Non-neoplastic Changes

Sex	Dose Group (% in Diet)			
	0	0.1	0.3	0.9
Males	11 (22%)	16 (33%)	30 (61%)**	26 (52%)**
females	14 (28%)	25 (51%)*	24 (48%)	24 (49%)

Results were obtained from Tables 11-2 (males) and 11-3 (females) in the study report. *p<0.05, **p<0.01 using a 2x2 contingency table.

Rats

The study authors noted many non-neoplastic changes in all rats sacrificed at 24 months including the controls. The study authors did not provide data on the non-neoplastic changes at the interim sacrifices that occurred at 6 and 12 months. The greatest number of changes observed were cardiac thrombosis, fatty liver, cerebral hemorrhage, hepatic bile duct proliferation, tubular casts, inflammatory nodules of the liver and ovary cysts. The only significant change was in the bile duct proliferation. Although there wasn't any difference in the total number of animals with proliferation, the treated animals had a greater number of animals with moderate proliferation than the controls. There were no changes in the total number of animals with non-neoplastic lesions with nearly all the rats displaying some change at 24 months. Because the study authors provided only data combining the animals that died or were sacrificed with the animals that survived the 24 months, further analysis of these data should be conducted to evaluate the toxicological effects. Therefore, a NOAEL could not be established.

Table 20. Number and Percent of Male Rats Displaying Bile Duct Proliferation

severity of bile duct proliferation	Dose (% in diet)				
	0	0.01	0.03	0.1	0.3
Total	27 (54%)	31 (63.3%)	36 (72%)	32 (66.7%)	36 (73.5%)
low +	26 (52%)	21 (42.9%)	23 (46%)	25 (52.1%)	26 (53.1%)
moderate ++	1 (2%)	10 (20.4%)**	12 (24%)**	7 (14.6%)	10 (20.4%)

Results were obtained from Table 22-2 (males) and 22-3 (females) in the study report. * p<0.05; ** p<0.01.

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b) Neoplastic

Mice

The study authors claim that although several of the mice developed tumors (i.e., leukemia, malignant lymphoma, pulmonary adenocarcinoma, and liver carcinoma) that there was no statistical differences between the incidences observed in the treated mice and the controls nor was there a dose-response relationship observed. While this appears correct, a proper assessment would include an analysis of animals sacrificed at termination separately from those that were sacrificed or died prematurely. The data, as summarized in the current report, combine all animals and thus preclude this type of analysis.

Rats

A dose-response relationship for leukemia was demonstrated in both males and females with the 0.3% group having the highest incidence (Table 21). The study authors claim that the incidence observed was within the range of previous reports and none of the treatment groups were statistically significantly increased over the control. Females also had a dose-response relationship for pituitary adenomas (Table 21). The study authors claim these are again within the spontaneous range observed in the literature and that none of the treatment groups were statistically significantly different from the controls. There was also a dose-response for endometrial polyps, but there was irregular distribution and the incidence was within historical control values (Table 21). The incidence of fibroademonas was high in comparison to the literature, but all treatment groups including the control were high. Our reviewer believes that these dose-response trends may be linked to treatment and the use of a higher dose may have better linked the treatment to tumor incidence. The study authors claim results to be within historical control data, but they do not provide the historical control data for comparison. Providing historical control data would help resolve whether an adequate high dose was tested by determining if the tumors are associated with the treatment or not. Also an analysis of animals that died during the study versus those sacrificed at termination, may reveal differences that are not apparent by combining all animals. Thus, the non-statistical significance of the incidence of leukemia may be a product of combining all the animals. A separate analysis is needed to fully assess the data.

Table 21. Neoplastic Lesions in the 24 month Group of Male and Female Rats

Tumor Type	Dose (% in diet)				
	0	0.01	0.03	0.1	0.3
Males					
Leukemia	7 (14%)	7 (14.3%)	7 (14%)	11 (22.9%)	14 (28.6%)
Females					
Leukemia[1]	2 (4.1%)	5 (10%)	6 (12.2%)	5 (10%)	9 (18.4)
pituitary (hypophysis) adenoma[1]	11 (22.4%)	16 (32%)	12 (24.5%)	19 (38%)	20 (40.8%)
endometrial polyps[2]	0	2 (4%)	5 (10.2%)	9 (18%)	7 (14.3%) *

Results were obtained from Table 20-2 (males) and Table 20-3 (females) of the study report. * $p<0.05$; ** $p<0.01$. [1]The study authors report a significant dose-response relationship with a $p< 0.05$ and present the results in Table 2.1 of the summary. [1]The study authors report a significant dose-response relationship with a $p< 0.01$ and present the results in Table 2.1 of the summary.

III. **DISCUSSION**

A. The study authors found several treatment related effects in both male and female rats and mice. Body weight was decreased in male mice administered 0.3 and 0.9%. In mice treated with 0.9% Zeomic, there was a significant decrease in RBC, hemoglobin, hematocrit, MCV and MCH parameters at all time points. There were significant decrease in hemoglobin, hematocrit, MCH, and MCHC of male mice at 24 months in the 0.1 and 0.3% groups and a decrease in RBC in the 0.3% group. There were also decreases at 0.3% in male mice, but they only occurred after 6 and 12 months. Female rats had a decrease in MCV at 6 and 12 months for treatment groups 0.1 and 0.3% which was not present at 24 months. There were moderate, but significant, increases in ALT and ALB at 24 hours in both male and female rat treated with 0.3%. TP and LDH activity in male and female rats had significantly reduced levels. Pigmentation in the liver, kidneys, pancreas, and stomach had a significant dose-response relationship in both males and females mice and rats for all time periods. Both male and female mice had an increase in renal cysts at 24 months. In addition male mice had an enlargement of the Langerhans' island and female mice had an increase in ovarian cysts.

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The study authors calculated a **NOAEL of 0.011 g/kg/day** using the 0.03% female rat data at 60 weeks. The study authors did not provide a LOEL. The ADI was calculated to be 6.6 mg/day and the AADI was 33 mg/kg.

B. Study deficiencies

General deficiencies for both mice and rats:

The study was not conducted under GLP. This deficiency cannot be corrected.

The study authors failed to provide some key data in the report that made it difficult for our reviewers to assess the results. The study authors were not careful in compiling the report. There are significant portions of data missing that were not noticed before the submission of the report as noted by the consecutive page numbers of the report. See the data validation review in Appendix A for a list of the missing data. Several times they made reference to weeks in the text when it should have been months and they made statements using the wrong month for comparison. These mistakes in the report are easily corrected.

The study authors specify that doses were selected using data from previous subchronic studies, but they did not provide specifics of these studies (only that a maximum dose of 5% was used, which caused no increase in tumor development, but caused a reduction in body weight). Providing the data from the subchronic study would assist the reviewers assess the dose selection.

The study authors did not mention tests to determine stability or homogenity of the compound in the food nor was there mention of samples to ensure that the proper concentration of the test material was present. Therefore, it is not possible to be certain that the ratios that the dose levels that were set were actually attained. Unless there are data available that the study authors failed to provide, this is a substantial deficiency as the study authors can not be certain as to the actual dose administered.

A light dark schedule of 14 hours light and 10 hours dark was used instead of the OPPTS guideline recommendation of 12 hours light and 12 hours dark. This would not be expected to have a significant impact the study results.

Mice Chronic

No urinalyses were performed. This is a deficiency that cannot be corrected.

The study authors did not perform any clinical chemistry on the mice. This is a significant deficiency that cannot be corrected.

Ophthamology exams were not performed. This is a deficiency that cannot be corrected.

Mice Carcinogenic

It is not apparent that a MTD was reached. Since male mice had an increase in food consumption, but a decrease in body weight it is possible that this was reached. However, in that the highest dose tested (0.9%) was calculated in the report to be in the range of the Limit Dose (704 to 1716), the Limit Dose (1000 mg/kg bw/day) was apparently approximated in males. The missing body weight data in females made it difficult to verify that the Limit Dose was approximated in females, although from the data that were available, the highest dose tested ranged from 617 to 1216.

The study authors did not examine several tissues (i.e., pituitary, bone marrow, spinal cord, and peripheral nerves) that are required under OPPTS Health Effects Test Guidelines 870.4300. The study authors did not list bone marrow under the tissues in Materials and Methods. It does not appear from the study report that these tissues were collected. The study authors also do not specify that they examined all gross lesions, just that tissues were prepared and examined and any abnormal results along with normal tissue were photographed. If the study authors have these tissues blocked in paraffin, but did not examine them, it would be possible to correct this deficiency. If no tissues were collected, it cannot be corrected.

The study authors report their cancer data using all the animals in the group. They did not present the data separately by those that died and those that lived to terminal sacrifice. This could have an effect on the interpretation of the results. This is a deficiency that can be corrected and is needed for our reviewers to adequately review the cancer data.

Rat Chronic

No urinalyses were performed. This is a deficiency that cannot be corrected.

Potassium and sodium were not analyzed. Since the study was conducted several years ago, this is not a deficiency that can be corrected.

Ophthamology exams were not performed.

Rat Carcinogenic

The study authors did not examine several tissues (i.e., pituitary, bone marrow, spinal cord, and peripheral nerves) that are required under OPPTS Health Effects Test Guidelines 870.4300. The study authors did not list bone marrow under the tissues in Materials and Methods. It does not appear from the study report that these tissues were collected. The study authors also do not specify that they examined all gross lesions, just that tissues were prepared and examined and any abnormal results along with normal tissue were photographed. If the study authors have these tissues blocked in paraffin, but did not

examine them it would be possible to correct this deficiency. If no tissue was collected, it cannot be corrected.

The study authors specify that there were several tumor-related dose responses in the rat, but that the levels did not statistically vary from the control and the numbers were low (i.e., 1/49 and 3/50). The authors also claim that even though there were statistical increases in tumor incidence above the control that these incidences were still within the incidences seen in historical controls, but they did not provide information on the historical control data. They could provide these data for further evaluation.

The effects observed at the high dose in the rat study are insufficient for concluding that an adequate dose was employed for carcinogenicity testing. The 1999 draft Carcinogenicity Assessment Guidelines state that "an adequate high dose would be one that produces some toxic effects without either unduly affecting mortality from effects other than cancer or producing significant adverse effects on the nutrition and health of the test animals." Alternately, if no target organ toxicity or physiological perturbation is observed, "an adequate high dose would be one that causes no more than 5%-10% reduction of body weight gain over the lifespan of the animals." The presentation of body weight data for the rats in this study makes it impossible to determine if there was this kind of body weight decrement, as a significant amount of body weight data are not presented for male rats. The data indicate that a higher dose could have been used in the rat study.

The study authors summarize their tumor data using all the animals in each group. They did not present the data separately for those that died on study and for those that lived to terminal sacrifice. This could have an effect on the interpretation of the carcinogenicity results. This is a deficiency that can be corrected and is needed for our reviewers to adequately review the cancer data.

APPENDIX A: DATA VALIDATION

Combined Chronic Toxicity/Carcinogenicity Study of Zeomic in Mice and Rats

I. ANIMAL GROUPS FOLLOWED THROUGHOUT THE STUDY

All mice and rat groups were reviewed in the study report, including all summary and referenced raw data. The groups reviewed are listed in the tables below.

Mice (for male and female groups)

Months Tested	Number of mice/sex/dose level	Dose level (%) sacrificed
3	5	control, 0.1, 0.3, 0.9
6	10	control, 0.1, 0.3, 0.9
12	10	control, 0.1, 0.3, 0.9
24	50	control, 0.1, 0.3, 0.9

Rats (for male and female groups):

Months Tested	Number of rats/sex/dose level	Dose level (%) sacrificed
6	10	control, .01, .03, 0.1, 0.3
12	10	control, .01, .03, 0.1, 0.3
24	10	control, .01, .03, 0.1, 0.3

No problems or discrepancies were noted in any of the data reviewed, however, our data validator did note missing as well as incorrectly ordered summary and raw data (mentioned below).

II. CRITICAL EFFECTS AND SPOT CHECKS

Means and standard deviations of the means were calculated using the referenced raw data in the study. The results were compared to corresponding summary tables in the study report. The summary tables that were validated are listed below.

Table 2 (p. 117): Changes in Blood Properties of Male and Female Mice.
Table 3 (p. 118): Mice Raised for 3, 6, and 12 Months.
Table 3-1 (p. 119): Dose-response Relationships on Various Neoplastic Changes in Mice raised for 24 Months.
Table 4 (p. 120): Changes of Blood Properties in Male and Female Rats.
Table 5-1 (p. 121): Incidence of Neoplastic Changes on Male and Female Rats Raised for 6 and 12

Months.
Table 5-2 (p. 122): Dose-Response Relationship on Various Neoplastic Changes of Male and Female Rats Raised for 24 Months.
Table 3-1 (p. 126): Outline of the Experimental Design (mice).
Table 3-2 (p. 127): Outline of the Experimental Design (rats).
Table 5-1 (p. 129): Changes in the Survival Rate of Male Mice.
Table 5-2 (p. 130): Changes in the Survival Rate of Female Mice.
Table 6-1 (p. 131): Changes in the Values of General Items of Observation in the Male Mice Through the Entire Observation Period.
Table 6-2 (missing first page): Changes in the Values of General Items of Observation in Male Mice During the Entire Observation Period.
Table 6-3 (missing first page): Changes in the Values of General Items of Observation in Female Mice During the Entire Observation Period.
Table 8-1 (p. 137): Changes in Organ Weights in the Male Mice.
Table 8-2 (p. 138): Changes in Organ Weights in the Female Mice.
Table 7 (p. 139): Changes in the hematological Profile in Male and Female Mice.
Table 9-1 (p. 140): Occurrence of Tumorous Changes in Male and Female Mice Observed for 3, 6, and 12 Months.
Table 9-2 (p. 141): Occurrence of Tumorous Changes in Male and Female Mice Observed for 24 Months.
Table 9-3 (p.142): Occurrence of Tumorous Changes in Female Mice Observed for 24 Months.
Table 10 (p. 143): Detection of Dose-Response Relations of Various Tumorous Changes in Mice Observed for 24 Months.
Table 11-1 (p. 144): Occurrence of Non-Tumorous Changes in Male and Female Mice Observed for 3, 6 and 12 Months.
Table 11-2 (p. 145): Occurrence of Non-Tumorous Changes in Male Mice Observed for 24 Months.
Table 11-3 (p. 146): Occurrence of Non-Tumorous Changes in Female Mice Observed for 24 Months.
Table 12 (p. 147): Detection of Dose-Response Relations of Various Non-Tumorous Changes in Mice Observed for 24 Months.
Table 13-1 (p. 148): Occurrence of Pigmentation in Male Mice.
Table 13-2 (p. 149): Occurrence of Pigmentation in Female Mice.
Table 14-2 (p. 150): Detection of Dose-Response Relations of Pigmentation in Male Mice.
Table 14-2 (p. 151): Detection of Dose-Response Relations of Pigmentation in Female Mice.
Table 15-1 (p. 152): Changes in the Survival Rate of Male Rats.
Table 15-2 (p. 153): Changes in the Survival Rate of Female Rats.
Table 16-2 (p. 154): Changes in the Results of General Observation Items in Male Rats Through the Entire Observation Period.
Table 16-2 (p. 157): Changes in the Results of General Observation Items in Female Rats Through the Entire Observation Period.
Table 17 (p. 161): Changes in the Hematological Profile in Male and Female Rats.
Table 18-1 (p. 162): Changes in the Values of Serum Chemical Parameters in the Male and Female Rats (items related to hepatic function).
Table 18-2 (p. 163): Changes in the Values of Serum Chemical Parameters in the Male and Female Rats (items related to lipid metabolism).
Table 18-3 (p. 164): Changes in the Values of Serum Chemical Parameters in the Male and Female Rats (pancreatic and renal functions and minerals).
Table 19-1 (p. 165): Changes in Organ Weights in the Male Rats.

Table 19-2 (p. 166): Changes in Organ Weights in the Female Rats.
Table 20-1 (p. 167): Occurrence of Tumorous Changes in Male and Female Rats Observed for 6 and 12 Months.
Table 20-2 (p. 168): Occurrence of Tumorous Changes in the Male Rats Observed for 24 Months.
Table 20-3 (p. 169): Occurrence of Tumorous Changes in the Female Rats Observed for 24 Months.
Table 22-2 (p. 170): Occurrence of Non-Tumorous Changes in the Male Rats Observed for 24 Months.
Table 22-3 (p. 172): Occurrence of Non-Tumorous Changes in the Female Rats Observed for 24 Months.
Table 23 (p. 173): Detection of Dose-Response Relations of Various Non-Tumorous Changes in the Male and Female Rats Observed for 24 Months.
Table 24-2 (p. 174): Occurrence of Pigmentation in Female Rats.
Table 25-1 (p. 175): Detection of Dose-Response Relations of Pigmentation in the Male Rats.
Table 25-2 (p. 176): Detection of Dose-Response Relations of Pigmentation in the Female Rats.

During this analysis, a few inconsistencies were detected. First, in Reference 8-3, Column "HCT", (p. 216), and in Reference 12-3, Column "Liver", (p. 246), data was not legible and therefore, the mean and standard deviations could not be validated.

Second, in Reference 9-1 (contd), Column T/H-C, (p. 219), a rounding error was detected for the standard deviation. The standard deviation was calculated as 0.118 rather than the reported value of 0.117. This rounding error is not thought to be significant.

Third, in Reference 12-3, Column "At the Beginning", (p. 248), the mean was calculated as 359.1 rather than the reported value of 359.9 and the standard deviation was calculated as 31.3 rather than the reported value of 30.53. This difference is not thought to be significant.

III. MISSING DATA

Summary data as well as raw data were found to be missing in the study report. This missing data was not noticed prior to being sent, as the page numbers are consecutive.

Summary Report:

- Table 6-2: "Changes in the values of general items of observation in female mice through the entire observation period" is missing data for weeks 0-17 and weeks 40-70.
- Table 16-1: "Changes in the results of general observation items in male rats through the entire observation period" is missing data for weeks 69-end.
- Table 24-1: "The occurrence of pigmentation in male rats" is missing.
- Table 21: "Dose-response relations of various tumorous changes in the male and female rats observed for 24 months" is missing.
- Table 22-1: "Occurrence of non-tumorous changes in male and female rats observed for 6 and 12 months" is missing.

Raw Data:

- Reference 2-4: "Results of hematological examinations in each female mouse observed for 24

months" is missing test compound concentrations of 0.1 and 0.9 % .

- Reference 4-4: "Organ weights (absolute) in all female mice observed in 24 months" is missing the control group. The data for female organ weight was not found on consecutive pages in the reference section and had to be sorted out.
- Reference 3-2: The organ weight for male mice is missing control data, 6 month data as well as the 0.3% test compound concentration for the 24 month test.
- Reference 5-2: "Histopathological findings in all male mice observed for 3 month" is missing.
- Reference 6-2: "Histopathological findings in all female mice observed for 3 month" is missing.
- Reference 7-1: "Results of hematological examinations in individual male rats observed for 6 months" is missing.
- Reference 7-3: "Results of hematological examinations in individual male rats observed for 24 months" is missing the text compound concentration 0.3%.
- Reference 9-2: "Results of clinical serum biochemical examinations in individual male rats observed for 12 months" is missing the control group, and 0.01 and 0.03% text compounds.
- Reference 10-1: "Results of serum chemical examination in female rats" is missing data for the control group, 0.01 and 0.03% test compounds for the 6 month study.
- Reference 10-2: "Results of serum chemical examination in female rats" is missing data for the control group, 0.01 and 0.03% text compounds for the 12 month study.
- Reference 10-3: "Results of the serum chemical examination in female rats" is missing the data for 0.3% test compound in the 24 month study.
- Reference 11-1: "Organ weight (absolute) in the individual male rats observed for 6 months" is missing the "At the Beginning" data column.
- Reference 11-2: "Organ weight (absolute) in the individual male rats observed for 12 months" is missing.
- Reference 12-3: "Organ weight (absolute) in the individual male rats observed for 24 months" is missing the 0.03 and 0.3% test compound data.
- Reference 13-3: "Histopathological findings in the individual male rats observed for 24 months" is missing the control group and 0.1% test compound data.

IV. FINDINGS

Findings from this data validation included some missing data. Also, the raw data was presented in a disorganized fashion, as it did not follow the order of the summary data and tables were scattered throughout the reference sections. Any inconsistencies between values listed in the study report and means and standard deviations determined by the data validator do not appear to be significant. However, we recommend that the laboratory be asked about these findings.

Liana Grossman

Liana Grossman, B.A.

06/04/02

Date