EPA Reviewer: John Allran, DABT
Risk Assessment Branch 6,
Existing Chemicals Risk Assessment Division, OPPT

Signature: Allran, John
Digitally signed by Allran, John
Date: 2024.03.12 16:56:38 -04'00'
Date:

EPA Secondary Reviewer: Colleen M. Rossmeisl, DVM
Risk Assessment Branch 2, Antimicrobials Division, OPP

Signature: COLLEEN ROSSMEISL
Digitally signed by COLLEEN ROSSMEISL
Date: 2024.03.12 13:14:22 -04'00'
Date:

DATA EVALUATION RECORD

TXR NO: N/A

STUDY TYPE: 2-Year Oral Toxicity drinking water study in rats; non-guideline

PC CODE: 043001 (formaldehyde), 043002 (paraformaldehyde)

DP BARCODE: N/A

TASK GROUP: N/A

TEST MATERIAL (PURITY): Paraformaldehyde (80% purity)

SYNONYMS: Formaldehyde, HCHO

CITATION: Tobe, M, Katsushi, N, and Kurokawa, Y. (1989) Chronic Toxicity Study on Formaldehyde Administered Orally to Rats. *Toxicology*, 56:79-86. Elsevier Scientific Publishers Ireland Ltd. Division of Toxicology, National Institute of Hygienic Sciences, Setagayaku, Tokyo, Japan. MRID 52279004

SPONSOR: None

EXECUTIVE SUMMARY: In a 2-year oral toxicity study, paraformaldehyde (80% a.i.) was administered to 20 Wistar rats/sex/dose group in drinking water at dose levels of 0, 0.02, 0.10, and 0.50% (equivalent to 0, 10, 50, and 300 mg/kg bw/day, respectively).

At 0.10% formaldehyde, treatment-related findings were limited to hyperkeratosis of the forestomach in 1/6 males at the 18-month interim sacrifice and in 1/8 females at termination at 24 months. Additionally, the study authors reported that total protein was significantly decreased in the surviving 0.10% males at 24 months, and dose-dependent decreases were observed in inorganic phosphorus in both sexes.

At 0.50%, the histopathology findings were more pronounced, and included incidences of erosions and ulcers in the forestomach and glandular stomach. In the forestomach, squamous cell hyperplasia, with and without hyperkeratosis, was observed, along with downward growth of

basal cells. Glandular hyperplasia of the fundic mucosa was noted along the limiting ridge. In the animals terminated at 12 months, the following treatment-related non-neoplastic lesions were observed in the stomach in rats treated with 0.5% formaldehyde (#affected/6 treated vs 0/6 controls): (i) squamous cell hyperplasia in the forestomach in males (6) and females (6); (ii) hyperkeratosis of the forestomach in males (4) and females (6); (iii) basal cell hyperplasia in the forestomach in males (4) and females (6); (iv) erosion/ulcer in the forestomach males (1) and females (2) and in the glandular stomach in males (6) and females (4); (v) submucosal cell infiltration in the forestomach in males (1) and females (2) and in the glandular stomach in males (3) and females (2); and (vi) glandular hyperplasia along the limiting ridge of the fundic mucosa in the glandular stomach in males (6) and females (4). It was noted that dilated gastric glands with clearly increased numbers of mucous neck cells were observed deep in the fundic mucosa.

This dose represents a frank effect level, with mortality observed as early as 9 days after start of treatment and reaching 45% in males and 55% in females by 12 months. All females in this dose group were dead by 21 months, and all males were dead by 24 months. The study authors stated that the "general condition of both male and female rats in the 0.50% group was poor", although no specific clinical signs of toxicity were reported. Significant decreases were observed in body weights, food consumption, and water consumption in both sexes. At 12 months, significant ($p \leq 0.05$) decreases were observed in total protein, albumin, and total cholesterol in both sexes. Blood urea nitrogen was significantly increased in males and females at this dose.

There were no treatment-related findings at 0.02%.

The LOAEL is 0.10% (equivalent to 50 mg/kg/day) based on hyperkeratosis in the forestomach in male and female Wistar rats. The NOAEL is 0.02% (equivalent to 10 mg/kg/day).

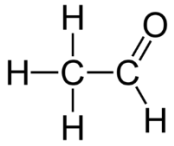
This 2-year oral toxicity study in the rat is **acceptable/non-guideline.**

COMPLIANCE: Good Laboratory Practice (GLP), Quality Assurance, and Data Confidentiality statements were not provided, as this study report was a peer-reviewed published journal article.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test Materials: Paraformaldehyde

Description	Crystalline paraformaldehyde
Lot/Batch #	Wako Pure Chemical Ind. Ltd., (Osaka, Japan)
Purity	80% AI
CAS of TGAI	50-00-0 (30525-89-4)
Structure	
Solvent Used	NA

2. Vehicle: Distilled Water

3. Test Animals:

Species	Rat
Strain	Slc:Wistar
Age/wt at study initiation	Weanling, 4 wks upon receipt; initial body weights not reported
Source	Shizuoka Laboratory Animals (Shizuoka, Japan)
Housing	Individually in suspended, stainless steel cages
Diet	Commercial F-1 (Funbashi Animal Farm Co. Ltd, Chiba), *ad libitum*
Water	Distilled water containing test concentrations of paraformaldehyde, *ad libitum*
Environmental Conditions	Temperature: 23 ± 2°C Humidity: 60 ± 10% Air changes: Not reported Photoperiod: Not reported
Acclimation period	Not reported

B. STUDY DESIGN

1. **In life dates:** Not reported

2. **Animal assignment:** Animals were assigned to the test groups noted in Table 1. The study report did not state that the assignment was random or if animals were stratified by body weight. The variation in male and female body weights at study initiation were not reported.

Table 1: Study Design (Formaldehyde in Drinking Water)

Test Group	Nominal Conc in Water (ppm)	Dose to animal (mg/kg-day)	Males (N)	Female (N)
Control	0	0	20	20
Low	0.02%	10	20	20
Mid	0.10%	50	20	20
High	0.50%	300	20	20

3. Dose selection rationale: Not provided.

4. Drinking water preparation and analysis: Drinking water test concentrations were prepared twice weekly throughout the study by dissolving the appropriate amount of crystalline paraformaldehyde in distilled water and mixing for 5 h at 80°C and then allowing to cool before providing to animals. No information was provided regarding concentration or stability analysis of formaldehyde in the drinking water.

Results:
Stability analysis: Not provided.

Concentration analysis: Not provided.

5. Statistics: Mortality was analyzed by life-table techniques. Hematology, clinical chemistry, and organ weight (absolute and relative) data were analyzed statistically using Student's t-test.

C. METHODS

1. Observations: Animals were observed daily for mortality and clinical signs of toxicity.

2. Body weight, food consumption, and water consumption: It was stated that body weights, food consumption, and water consumption were measured once weekly or biweekly.

3. Test substance intake: The study authors reported that achieved intake was calculated from the mean water consumption and body weight data.

5. Hematology and clinical chemistry: Blood was collected from each animal prior to termination at 12-, 18-, and 24-months for hematology and clinical chemistry analysis. It was not stated whether animals were fasted prior to blood collection. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*		Leukocyte differential count*
X	Hemoglobin (HGB)*		Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*		Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*		Mean corpusc. volume (MCV)*
	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for combined chronic and carcinogenicity studies based on Guideline 870.4300.

b. Clinical chemistry:

	ELECTROLYTES		OTHER
	Calcium	X	Albumin*
	Chloride		Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
	Potassium*		Globulins
	Sodium*		Glucose*
	ENZYMES (more than 2 hepatic enzymes eg., *)		Total bilirubin
X	Alkaline phosphatase (ALP)*	X	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for combined chronic and carcinogenicity studies based on Guideline 870.4300.

6. **Urinalysis*:** Urinalysis was not conducted

7. **Sacrifice and pathology**: At 12 months and at 18 months, 6 rats from each dose group were randomly selected for termination, and all surviving animals were killed at 24 months. The method for euthanasia was not reported. All animals that died and those sacrificed on schedule were subjected to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. Samples of tissues were preserved in 10% buffered formalin, embedded in parrafin, sectioned, and stained with hematoxylin and eosin for histopathological examination. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue		Aorta*	XX	Brain*+
	Salivary glands*	XX	Heart*+		Peripheral nerve*
	Esophagus*		Bone marrow*		Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*+		Eyes (optic nerve)*
X	Jejunum*		Thymus*+		**GLANDULAR**
X	Ileum*			XX	Adrenal gland*+
X	Cecum*		**UROGENITAL**		Lacrimal gland
X	Colon*	XX	Kidneys*+		Parathyroid*
X	Rectum*		Urinary bladder*	XX	Thyroid*
XX	Liver*+	XX	Testes*+		**OTHER**
	Gall bladder (not rat)*		Epididymides*+		Bone (sternum and/or femur)
	Bile duct (rat)		Prostate*		Skeletal muscle
X	Pancreas*		Seminal vesicles*		Skin*
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
	Trachea*	X	Uterus*+ with cervix		
XX	Lung*++		Mammary gland*		
	Nose*				
	Pharynx*				
	Larynx*				
	Nasal cavity				

* Required for combined chronic/carcinogenicity studies based on Guideline 870.4300.
+Organ weight required in combined chronic/carcinogenicity studies.
++Organ weight required if inhalation route.

II. RESULTS

A. OBSERVATIONS

1. **Clinical signs of toxicity:** The study authors stated that the "general condition of both male and female rats in the 0.50% group was poor". However, no specific clinical signs of toxicity were reported.

2. **Mortality:** It was reported that mortality was observed as early as 9 days after start of treatment in the 0.50% formaldehyde group. Mortality reached 45% in males and 55% in females by 12 months (Table 2). All females in this dose group were dead by 21 months, and all males were dead by 24 months. The deaths at 0.02% and 0.10% could not be attributed to treatment, as they were low in incidence and/or unrelated to dose.

TABLE 2. Mortality (%) in rats exposed to formaldehyde in drinking water for up to 2 years [a]				
Months	**Dose (%)**			
	0	**0.02**	**0.10**	**0.50**
Males				
3	0	0	0	10
6	0	0	0	15
9	0	0	0	20
12	0	0	0	45
15	0	0	0	67
18	0	7.1	0	67
21	0	20.4	0	78
24	12.5	46.9	0	100
Females				
3	0	0	0	25
6	0	0	0	30
9	0	0	0	30
12	0	0	0	55
15	0	7.1	0	55
18	14.3	7.1	14.3	70
21	28.6	20.4	14.3	100
24	28.6	33.7	14.3	100

[a] Data obtained from Table I on p. 82 in the study report. % by Sachs' life-table analysis.

B. <u>BODY WEIGHT AND FOOD AND WATER CONSUMPTION</u>: The study authors stated that body weight gains, food consumption, and water consumption were significantly decreased in males and females in the 0.50% formaldehyde group. These data were not presented numerically in tables but were depicted graphically in Figures 1 and 2 of the study report, inserted below.

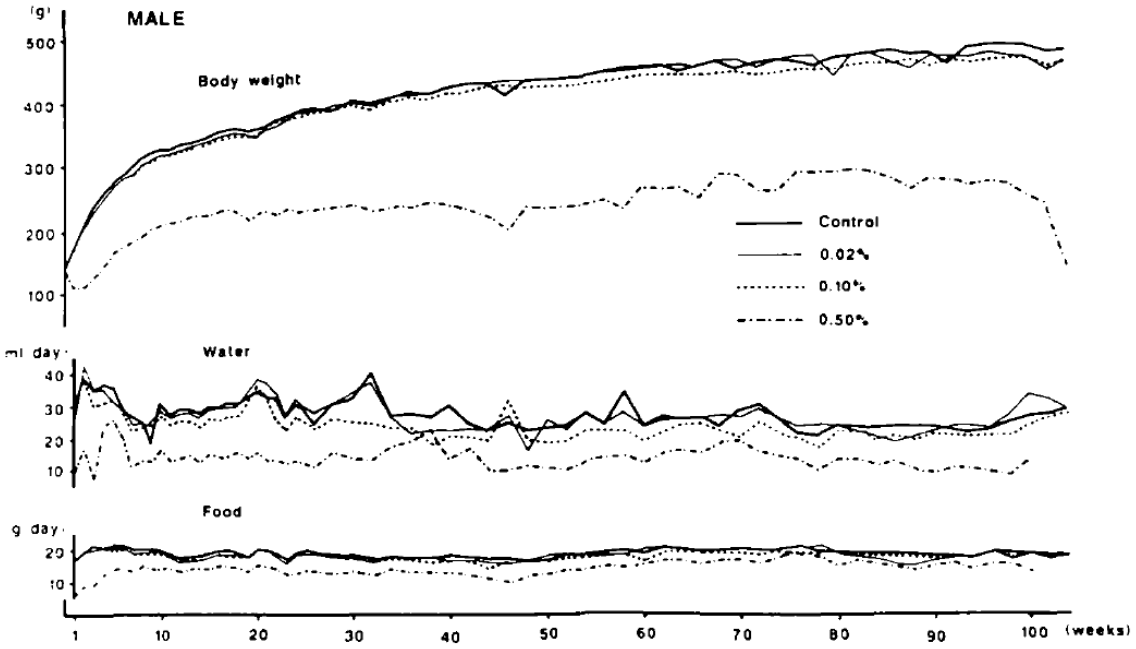


Fig. 1. Changes in the body weight and intake of water and food in male rats given formaldehyde orally.

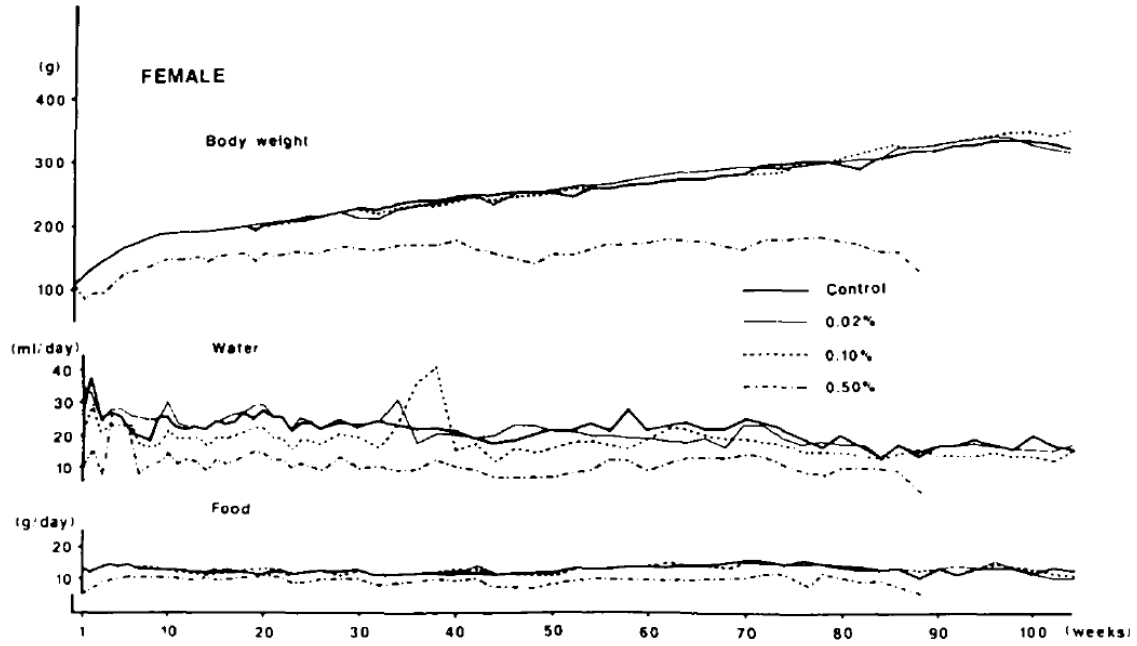


Fig. 2. Changes in the body weight and intake of water and food in female rats given formaldehyde orally.

3. Test substance intake: The study authors reported that the achieved doses presented in the study report were calculated from the mean water consumption and body weight data (Table 1). No additional data was provided to confirm the reported doses. Test substance intake was not calculated separately for males and females.

D. OPHTHALMOSCOPIC EXAMINATION: Not conducted.

E. BLOOD ANALYSES

1. Hematology: The study authors reported that hemoglobin and the number of erythrocytes were decreased in the treated rats throughout the study but that these decreases were not dose-related. No data were presented in the study report.

2. Clinical chemistry: Selected clinical chemistry parameters at the 12-month interim sacrifice are included in Table 3. At 12 months, significant ($p \leq 0.05$) decreases were observed in total protein, albumin, and total cholesterol in both sexes in 0.50% treatment group. Blood urea nitrogen was significantly increased in males and females at this dose. No other treatment-related findings in clinical chemistry were evident at 12 months. AST and ALT were significantly ($p \leq 0.05$) decreased in the 0.50% males; however, decreases in these liver enzymes are not considered adverse. AST and ALT were significantly decreased ($p \leq 0.05$) in the females at 0.10% formaldehyde; however, in addition to not being adverse, these differences were not dose related, with no significant differences from controls observed at 0.50%.

The study authors reported that total protein was significantly decreased in the surviving 0.10% males at 24 months, and dose-dependent decreases were observed in inorganic phosphorus in both sexes. However, no data were presented.

TABLE 3. Selected clinical chemistry findings in rats exposed to formaldehyde in drinking water for up to 2 years – Interim 12-month sacrifice [a]				
Parameter	**Dose (%)**			
	0	**0.02**	**0.10**	**0.50**
Males				
Total protein (g/dL)	7.9 ± 1.3	7.7 ± 1.7	7.2 ± 0.5	5.9 ± 0.6** (↓25%)
Albumin (g/dL)	5.0 ± 0.5	4.8 ± 1.0	4.5 ± 0.3	4.2 ± 0.4** (↓16%)
Total cholesterol (mg/dL)	130.5 ± 11.5	113.6 ± 29.3	115.9 ± 16.4	80.2 ± 12.4** (↓39%)
Blood Urea Nitrogen (mg/dL)	18.5 ± 2.5	20.3 ± 2.9	20.0 ± 1.4	23.6 ± 3.3* (↑28%)
Females				
Total protein (g/dL)	8.0 ± 0.5	7.8 ± 0.6	7.8 ± 0.5	5.8 ± 0.8** (↓28%)
Albumin (g/dL)	5.5 ± 0.8	5.6 ± 0.8	5.4 ± 0.9	4.2 ± 0.6* (↓24%)
Total cholesterol (mg/dL)	178.6 ± 7.4	174.2 ± 18.0	178.0 ± 14.9	109.4 ± 10.8** (↓39%)
Blood Urea Nitrogen (mg/dL)	16.2 ± 2.6	18.4 ± 1.9	18.4 ± 2.9	31.6 ± 16.6* (↑95%)

[a] Data obtained from Table II on p. 83 in the study report; n=6. Percent difference from controls was calculated by the reviewers from group means and included in parentheses.
* Statistically different from the control group at $p \leq 0.05$.
** Statistically different from the control group at $p \leq 0.01$.

F. URINALYSIS: Not conducted.

G. SACRIFICE AND PATHOLOGY

1. Organ weight: The study authors reported that there were no dose-related significant changes in absolute or relative organ weights. However, no data were presented.

2. Gross pathology: No data were presented for macroscopic findings.

3. Microscopic pathology

a. Non-neoplastic findings: In the animals terminated at 12 months, the following treatment-related non-neoplastic lesions were observed in the stomach in rats treated with 0.50% formaldehyde (Table 4; #affected/6 treated vs 0/6 controls): (i) squamous cell hyperplasia in the forestomach in males (6) and females (6); (ii) hyperkeratosis of the forestomach in males (4) and females (6); (iii) basal cell hyperplasia in the forestomach in males (4) and females (6); (iv) erosion/ulcer in the forestomach males (1) and females (2) and in the glandular stomach in males (6) and females (4); (v) submucosal cell infiltration in the forestomach in males (1) and females (2) and in the glandular stomach in males (3) and females (2); and (vi) glandular hyperplasia along the limiting ridge of the fundic mucosa in the glandular stomach in males (6) and females (4). It was noted that dilated gastric glands with clearly increased numbers of mucous neck cells were observed deep in the fundic mucosa.

TABLE 4. Microscopic findings in rats exposed to formaldehyde in drinking water for up to 2 years – Interim 12-month sacrifice [a]								
Microscopic finding	**Male**				**Female**			
Dose (%)	**0**	**0.02**	**0.10**	**0.50**	**0**	**0.02**	**0.10**	**0.50**
Forestomach								
Squamous cell hyperplasia	0	0	0	6	0	0	0	6
Hyperkeratosis	0	0	0	4	0	0	0	6
Basal cell hyperplasia	0	0	0	4	0	0	0	6
Erosion/ulcer	0	0	0	1	0	0	0	2
Submucosal cell infiltration	0	0	0	1	0	0	0	2
Glandular stomach								
Glandular hyperplasia	0	0	0	6	0	0	0	4
Erosion/ulcer	0	0	0	6	0	0	0	4
Submucosal cell infiltration	0	0	0	3	0	0	0	2

[a] Data obtained from Table III on p. 84 in the study report; n=6

At 0.10%, forestomach hyperkeratosis was observed in 1/6 males at the 18-month sacrifice and in 1/8 females at the 24-month termination. However, there were no mucosal lesions of the glandular stomach at this dose.

At 0.02%, no treatment-related microscopic lesions were noted in the forestomach or glandular stomach.

b. **Neoplastic findings**: The study authors reported that various types of tumors were observed in organs such as the pituitary, thyroid, testis, adrenals, mammary gland, and skin. However, none of these tumors was attributed to treatment because they: occurred in all dose groups, including the control, in a manner unrelated to dose; were not significantly increased over concurrent controls; and/or were histologically similar to tumors arising spontaneously in this strain of rat. No additional data was provided on these results.

III. DISCUSSION AND CONCLUSIONS

A. **INVESTIGATORS' CONCLUSIONS:** The investigators concluded that the LOAEL was 0.10% formaldehyde (equivalent to 50 mg/kg-day) based on histopathology lesions in the forestomach (e.g., hyperkeratosis) in 1 rat/sex exposed in drinking water for up to 2 years at this dose. The NOAEL is 0.02% formaldehyde (equivalent to 10 mg/kg-day). At 0.50% formaldehyde (equivalent to 300 mg/kg-day), mortality was observed, with approximately half of the animals dead by 12 months and all of the animals dead by 24 months. Additionally at the high dose, decreased body weights, water consumption, and food consumption were observed, along with higher incidence and severity of histopathological lesions in the forestomach and glandular stomach.

B. **REVIEWER COMMENTS:** In this 2-year oral toxicity study, paraformaldehyde (80% a.i.) was administered to 20 Wistar rats/sex/dose group in drinking water at dose levels of 0, 0.02,

0.10, and 0.50 (equivalent to 0, 10, 50, and 300 mg/kg bw/day, respectively).

At 0.50%, the histopathology findings were more pronounced, and included incidences of erosions and ulcers in the forestomach and glandular stomach. In the forestomach, squamous cell hyperplasia, with and without hyperkeratosis, was observed, along with downward growth of basal cells. Glandular hyperplasia of the fundic mucosa was noted along the limiting ridge. In the animals terminated at 12 months, the following treatment-related non-neoplastic lesions were observed in the stomach in rats treated with 0.50% formaldehyde (#affected/6 treated vs 0/6 controls): (i) squamous cell hyperplasia in the forestomach in males (6) and females (6); (ii) hyperkeratosis of the forestomach in males (4) and females (6); (iii) basal cell hyperplasia in the forestomach in males (4) and females (6); (iv) erosion/ulcer in the forestomach males (1) and females (2) and in the glandular stomach in males (6) and females (4); (v) submucosal cell infiltration in the forestomach in males (1) and females (2) and in the glandular stomach in males (3) and females (2); and (vi) glandular hyperplasia along the limiting ridge of the fundic mucosa in the glandular stomach in males (6) and females (4). It was noted that dilated gastric glands with clearly increased numbers of mucous neck cells were observed deep in the fundic mucosa.

This dose represents a frank effect level, with mortality observed as early as 9 days after start of treatment and reaching 45% in males and 55% in females by 12 months. All females in this dose group were dead by 21 months, and all males were dead by 24 months. The study authors stated that the "general condition of both male and female rats in the 0.50% group was poor", although no specific clinical signs of toxicity were reported. Significant decreases were observed in body weights, food consumption, and water consumption in both sexes. At 12 months, significant ($p \leq 0.05$) decreases were observed in total protein, albumin, and total cholesterol in both sexes and are likely related to the decreased food consumption and body weights in these animals. Blood urea nitrogen was significantly increased in males and females at this dose.

At 0.10% formaldehyde, treatment-related findings were limited to hyperkeratosis of the forestomach in 1/6 males at the 18-month interim sacrifice and in 1/8 females at termination at 24 months. Additionally, the study authors reported that total protein was significantly decreased in the surviving 0.10% males at 24 months, and dose-dependent decreases were observed in inorganic phosphorus in both sexes. Although lesions only occurring in the forestomach may be less relatable to human pathology based on the lack of a forestomach in humans, changes noted here are similar to those found in other studies at similar concentrations that were also noted in the glandular stomach (Til, 1986). In addition, forestomach lesions observed in this study may be relatable to potential esophageal effects in humans (histopathological examination of the esophagus was not conducted in this study). At the next highest dose level (0.50%), much more severe effects were noted, including 100% mortality by the end of the study. For these reasons, the LOAEL is conservatively based on the effects noted at the 0.10% dose level.

There were no treatment-related findings at 0.02%.

The LOAEL is 0.10% (equivalent to 50 mg/kg/day) based on hyperkeratosis in the

forestomach in male and female Wistar rats. The NOAEL is 0.02% (equivalent to 10 mg/kg/day).

This 2-year oral toxicity study in the rat is **acceptable/non-guideline.**

C. STUDY DEFICIENCIES: The following study deficiencies were noted:

- Concentration and stability of the formaldehyde in the drinking water were not reported. However, the frequency of the test solution preparation (twice weekly in drinking water) provides some confidence in the achieved dose.
- The study authors reported that the achieved doses presented in the study report were calculated from the mean water consumption and body weight data. However, test substance intake was not calculated separately for males and females and therefore likely represents an average. No specific information was provided by the author via tabulated data, and dose levels could not be confirmed or recalculated.
- Numerical (tabular) data were not included for body weights, food consumption, or water consumption. However, this deficiency does not impact the acceptability of the study because these data were presented in a graph, and the decreases at the high dose represented a frank effect level, associated with high mortality. Data presented in the graph indicate that values for these parameters at the low and mid dose were likely comparable to controls.
- No incidence data were reported for clinical signs. However, this deficiency does not impact the acceptability of the study because the study authors stated that the "general condition of both male and female rats in the 0.50% group was poor", and this dose level represented a frank effect, so the details of specific clinical observations are less important than the mortality observed at that dose. Further, the reviewers assume that any specific clinical signs of toxicity observed at lower doses would have been reported by the study authors.
- No incidence data were reported for hematology. However, this deficiency does not impact the acceptability of the study because the study authors reported in text that there were decreases in hemoglobin and number of erythrocytes in the treated rats but that these decreases were not dose-related. Thus, the reviewers assume that any dose-related changes in hematology would have at least been described in text.
- No incidence data were reported for gross pathology. However, this deficiency has negligible impact on the acceptability of the study because incidences of histopathology findings in the stomach were reported.