THESIS

This is to certify that the

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THE EFFECTS OF SODIUM HYPOCHLORITE ON THE GROWTH AND REPRODUCTION OF MINK (MUSTELA VISON)

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THE EFFECTS OF SODIUM HYPOCHLORITE ON THE GROWTH AND REPRODUCTION OF MINK (MUSTELA VISON)

By

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ABSTRACT

THE EFFECTS OF SODIUM HYPOCHLORITE ON THE
GROWTH AND REPRODUCTION OF MINK (MUSTELA VISON)

By

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Feed and water consumption studies were done to ascertain the levels at which mink could tolerate sodium hypochlorite (NaOCl) in their feed or drinking water. Mink were more tolerant of NaOCl in their feed than in their drinking water. Feed consumption was significantly reduced at 3200 ppm NaOCl while water consumption was reduced at 200 ppm.

NaOCl was added to the drinking water of mink at 25, 50, 100, and 200 ppm and to the feed at 100 ppm to determine the effects on growth and reproduction. The addition of NaOCl to the drinking water or feed at the levels indicated did not have a significant effect (beneficial or detrimental) on the growth or reproduction of mink.

Aerobic plate counts of mink feed treated with 100 ppm NaOCl indicated that this compound did not significantly reduce the rate of bacterial growth over a 24 hour period.

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TABLE OF CONTENTS

LIST OF TABLES

Page

INTRODUCTION

Sodium hypochlorite is a compound that has wide industrial and agricultural usage. It is a major component of some commercial bleaches and is commonly used as a disinfectant. It is effective against a variety of microorganisms, including Pseudomonas. Recommended levels of chlorine necessary for Pseudomonas inactivation in mice are in the range of 12-16 ppm (Simmons and Brick, 1969).

Recent research conducted by Cunningham (1980) has shown that sodium hypochlorite (NaOCl) has a growth stimulating effect on rats and guinea pigs when fed at low concentrations. The mode of action of dietary supplemental NaOCl is not known. It may act as an antibiotic in counter-acting the effects of low-level gastrointestinal infective agents and/or it may decrease the non-pathogenic flora of the digestive tract that compete with the animal for food (Cunningham, 1980).

Other evidence indicates that at higher chlorine levels (25-30 ppm), there is an adverse effect on the macrophage system (an important defense mechanism, which functions to eliminate microbial pathogens and neoplasms of mice) (Fidler, 1977). Also, chlorite (ClO_2^-), which contains one more oxygen than hypochlorite (ClO^-), was shown by Moore et al. (1980) to decrease

the conception rate of A/J mice and of retarding the growth rate of A/J pups through weaning when administered via drinking water at 100 ppm. It may be that hypochlorite acts in the same manner as chlorite.

Since sodium hypochlorite is relatively inexpensive and is frequently used as a disinfectant by mink ranchers, this study was conducted to investigate the effects this compound might have on mink. This investigation was conducted to ascertain the effects of NaOCl on adult mink, as well as on the kits whelped and nursed by exposed females. A feed and water consumption study was conducted to determine what levels of NaOCl in the feed or the water mink would tolerate and to select appropriate levels to be used in the growing and reproduction trial. An attempt was made to determine if the addition of NaOCl to the feed might decrease the rate of bacterial growth (spoilage) in conventional mink diets.

LITERATURE REVIEW

HISTORY

Sodium hypochlorite's main use today and in the past is as a deodorizer and disinfectant. Although hypochlorites were used for textile bleaching in the late 1700's, their deodorizing and disinfecting properties were not recognized until the first half of the nineteenth century (Drychdala, 1983). In 1825, Lebarraque, a French druggist, recommended the use of hypochlorites as deodorants and antiputrifactives for corpses in the Paris morgue (Bernarde, 1970). Alcock, in 1827, suggested the use of chlorine as a disinfectant. In 1846, Semmelweis, a Viennese obstetrician, recommended that chloride of lime solutions (hypochlorites) be used for the washing of hands of medical personnel between the examinations of patients (Brock, 1961). This brought about the control of puerperal (childbirth) fever in his clinic. Koch, a German bacteriologist, in 1881, demonstrated that pure cultures of bacteria could be destroyed by the use of hypochlorites (Brock, 1961) and in 1886, the American Public Health Association issued a favorable report on the use of hypochlorites as disinfectants (Hadfield, 1957).

Traube, in 1894, established the purifying and disinfecting properties of hypochlorites in water treatment. They were first

used in this country for water treatment in 1908 by Mr. G.A. Johnston at the Chicago stockyards. The use of chlorine as a water purifier has increased ever since. The use of chlorine as a disinfectant increased tremendously since Dakin introduced his Dakin's solution (a solution of 0.45 to 0.50% NaOCl) in 1915 for the disinfection of wounds. This solution was used extensively during World War I (Drychdala, 1983).

USAGE AND EFFECTIVENESS

Since the hypochlorites are a proven and powerful germicide, a deodorizer, nonpoisonous to man at use concentrations, economical to use, free of poisonous residuals, colorless and easy to handle, they are used widely in many industries (Lesser, 1949). Hypochlorites are used as sanitizers in households, hospitals, schools and public buildings and as disinfectants in restaurants, food processing plants, dairies, canneries, breweries, wineries, and beverage bottling plants. They are also used for the treatment of drinking water, swimming pool water, spas, sewage and waste water (Drychdala, 1983). Some of the concentrations at which chlorine disinfectants are used in these various industries are given in Appendix A. Appendix B shows the effectiveness of chlorine compounds as disinfectants.

MODE OF ACTION - CHLORINE DISINFECTION

The mechanism by which chlorine (specifically sodium hypochlorite) acts as a disinfectant is not fully known. Sodium

hypochlorite, when added to water, undergoes the following reaction:

$$NaOCl + H_2O \longrightarrow HOCl + Na^+ + OH^-$$

Hypochlorous acid (HOCl) has been shown to be the main compound responsible for the bactericidal action of NaOCl (Andrews and Orton, 1904; Baker, 1959). HOCl exists in equilibrium with its ionized form ^-OCl according to its ionization constant. The dissociation of HOCl

$$HOCl \rightleftharpoons H^+ + {}^-OCl \quad 6.8 \times 10^{-8} \text{ (Holst, 1940)}$$

to ^-OCl depends primarily on the pH. As the pH increases the amount of HOCl decreases and the germicidal properties of NaOCl decrease and vice-versa. This is illustrated by the graph in Appendix C showing pH versus HOCl and ^-OCl. This again indicates that HOCl is mainly responsible for the germicidal action of NaOCl and is a more powerful disinfectant than ^-OCl. Fair et al. (1948) and Morris (1926) showed theoretically that the ^-OCl ion possesses about 1/80 of the bacteriocidal action of HOCl.

How HOCl produces the bacteriocidal effect is a matter of debate. Baker (1926) suggests that chlorine binds with proteins of the bacterial cell membrane, forming N-chloro compounds, which interferes with cell metabolism and leads to eventual death of the organism. Rudolph and Levine (1941), stated that an active germicidal ingredient penetrates the bacterial cell and forms toxic N-chloro compounds when it contacts the cell protoplasm.

The enzyme trace substance theory of Green and Stumpf (1946) postulated that chlorine must inhibit some key enzymatic reactions within the cell, since it is effective at such low concentrations. This was later confirmed by Knox et al. (1948), who showed that chlorine in bacteriocidal amounts or less inhibit various sulfhydryl enzymes and other enzymes sensitive to oxidation. Inhibition of the essential enzymes is irreversible and causes death to the cell.

TOXICITY

Sodium hypochlorite is generally considered safe for higher animals and man at use concentrations. Upon ingestion of excessive quantities of NaOCl, corrosion of mucous membranes, gastric perforations, laryngeal edema, methemoglobinemia, and death can occur. Inhalation of large amounts of NaOCl can cause severe bronchial irritation and pulmonary edema and prolonged skin contact may result in vesicular eruptions (Anonymous, 1976).

The lethal dose of NaOCl give by intra-peritoneal injection to mice and guinea pigs has been reported to be between 100 to 150 mg/kg body weight (Taylor and Austin, 1918), although Cunningham (1980) found no signs of toxicity when NaOCl was given orally to mice in milk (gavage) at concentrations up to 200 mg of available chlorine/kg body weight/daily for 2 weeks.

High concentrations of free chlorine (2,000 to 10,000 ppm), when added to cake flour, have been found to be toxic to rats.

The most common signs of toxicity were reduced growth rate and enlarged liver, kidney and heart (Cunningham et al., 1977; Cunningham and Lawrence, 1978). Hulan and Proudfoot (1982) also found increased mortality and decreased feed conversion, but a decrease in organ weights (liver, kidney, heart and testes) in chicks given 600 or 1200 ppm available chlorine in their drinking water.

Furukawa et al. (1980) noted a tendency towards dehydration in rats administered water containing up to 4% NaOCl (4000 ppm) for 14 days but observed no clinical signs of toxicity. Also, no pathological changes were noticed in rats treated with 0.4% NaOCl (400 ppm) in their water for 92 days. Cunningham (1980) found no evidence of toxicosis in rats administered NaOCl at up to 1000 ppm of available chlorine in their drinking water. Druckery (1968) found no effects on fertility, growth, hematologic parameters, or histology of organs in rats administered water containing 100 ppm available chlorine over their life span. These studies indicate that at low concentrations (< 1000 ppm available chlorine) sodium hypochlorite is relatively non-toxic. Sodium hypochlorite is seldom used at concentrations above 1000 ppm available chlorine, so toxicity should not be a problem.

Possible potential hazards may, however, arise when sodium hypochlorite is accidently used in conjunction with other compounds. The addition of acids to hypochlorites can lead to the production of deadly chlorine gas. NaOCl can also react with

formaldehyde to form the potentially dangerous lung carcinogen bis-chlormethyl ether with a threshold limit value of 1 part per billion (Gamble, 1977). NaOCl has also been shown to be a cocarcinogen when used with 4-nitroquinoline 1-oxide (Hayatsu et al., 1971). Chloroform, another carcinogen, has been shown to be produced in vivo after the ingestion of NaOCl (Vogt et al., 1979). Likewise, dichloroacetonitrile, which was shown to have mutagenic activity in the Ames biassay (Simmon et al., 1977), was found to be formed in vivo after the ingestion of NaOCl (Mink et al., 1983).

RELATED COMPOUNDS

Chlorine has long been used for the primary disinfection of drinking water in the United States. Recently, it has been shown that the treatment of drinking water with chlorine results in the formation of trihalomethanes, such as chloroform, which has been shown to be carcinogenic in mice and rats (Rook, 1974; Bellar et al., 1974; Anonymous, 1976). Chlorine dioxide has been suggested as a replacement for chlorine in water disinfection. Chlorine dioxide (ClO_2) and the by-products of its use, chlorite (ClO_2^-) and chlorate (ClO_3^-), are more oxidized forms of hypochlorite (OCl^-) and hence are more potent oxidizers.

Studies using rats, mice, and chickens treated with ClO_2, ClO_2^-, and ClO_3^- in drinking water have shown alterations of hematologic parameters in all species tested (Couri et al., 1982). The effects were usually dose related with marked changes

occurring at concentrations above 100 ppm. These treated groups showed alterations in erythrocyte morphology and osmotic fragility, and a dose related decrease of blood glutathione content. A recent study with rats showed that ClO_2, ClO_2^-, and ClO_3^- increased the turnover of cells of the gastrointestinal mucosa and inhibited DNA synthesis in several organs, including the testes, which could possibly cause reproductive failure in the males (Couri et al., 1982). Hypochlorites generated from the use of chlorine disinfectants have shown none of these adverse characteristics.

MINK EXPERIMENTS

FEED AND WATER CONSUMPTION OF NaOCl

Purpose

This trial was conducted to determine the concentrations of sodium hypochlorite that mink would tolerate in their feed or drinking water. These limits were then used to select the levels to be used during the growth and reproduction experiment.

Materials and Methods

Nine standard dark male mink were used on the water consumption trial and eight on the feed consumption experiment. The mink were housed indoors in wire cages that measured 61.5 cm length x 41.0 cm width x 36.0 cm height. The mink were allowed to acclimate for a period of five days so that animals on the water consumption study could adjust to drinking water from water bottles and those on the feed study could adjust to their new feeding regime. Both experiments were conducted as a simple (complete block) crossover design, that is, each animal received each NaOCl treatment level including control for two days, followed by a day of no treatment. Treatment means for both experiments were compared used Dunnett's one-sided t-test (Gill, 1978).

Mink on the water consumption trial were given NaOCl-treated water in "laboratory-type" inverted glass bottles. These bottles were used to insure an accurate measurement of water consumption and to minimize water evaporation which could increase the concentration of sodium hypochlorite. The NaOCl-treated water was made fresh daily and water consumption measured daily. Sodium hypochlorite[1] was added to the drinking water at the following concentrations (ppm): 0, 25, 50, 100, 200, and 400.

Mink on the feed consumption study were given feed twice daily for a two hour period (8 to 10 a.m. and 3 to 5 p.m.). The amounts of feed consumed during each two hour period were summed to give the total feed consumption per day. This feeding regime was used because sodium hypochlorite is known to react with the organic matter in the feed, thus decreasing the concentration of NaOCl over time. If the mink did not consume the feed initially they might consume the feed later after the NaOCl concentration decreased thus yielding false feed consumption values. NaOCl-treated feed was made fresh prior to each two hour feeding period. Sodium hypochlorite was added to the mink diet at the following concentrations (ppm), 0, 200, 400, 800, 1600, and 3200.

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[1]Laboratory grade sodium hypochlorite (4-6% NaOCl), Fisher Scientific Co., Chemical Manufacturing Division, Fairlawn, NJ 07410. Solution contains 4-6% available chlorine.

Results

Water consumption by the mink was not affected at concentrations up to 50 ppm NaOCl. Above 50 ppm there was a dose-response relationship, as the concentration of NaOCl increased, water consumption decreased. Concentrations of NaOCl of 200 ppm and greater resulted in a significant reduction in water consumption, when compared with that of the control group (Table 1).

As shown in Table 2, feed consumption of the mink given NaOCl-treated feed was not affected at concentrations up to 1600 ppm. At 3200 ppm NaOCl, feed consumption was reduced significantly when compared to that of the control group.

Discussion

The sodium hypochlorite solution used for these studies contained 4-6% NaOCl. This product yielded 4-6% available chlorine[2], so for those trials the concentration of NaOCl and available chlorine was equivalent.

The results of this experiment indicated that mink are less tolerant of chlorine in their drinking water than are chicks or rats. Chicks tolerated up to 300 ppm chlorine in their drinking water (Hulan and Proudfood, 1982), while rats showed no decrease

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[2]Available chlorine is a measurement of the oxidizing capacity and is expressed in the terms of the equivalent amount of elemental chlorine.

Table 1. Water consumption of male mink given various concentrations of NaOCl-treated drinking water for 2 days.

	n	Concentration of supplemental NaOCl (ppm)					
		0 (Control)	25	50	100	200	400
Water consumption[1] (ml/day)	9	87.0 ± 4.9	83.4 ± 4.9	83.3 ± 4.9	71.7 ± 4.9	66.3 ± 4.9[a]	37.5 ± 4.9[a]

[1]Mean ± standard error.

[a]Significantly different from control ($P < 0.01$) - Dunnett's one-sided t-test.

Table 2. Feed consumption of male mink given various concentrations of NaOCl-treated feed for 2 days.

		Concentration of supplemental NaOCl (ppm)					
	n	0 (Control)	200	400	800	1600	3200
Feed consumption[1] (gm/day)	8	124.1 ± 3.9	126.3 ± 3.9	125.0 ± 3.9	127.9 ± 3.9	122.8 ± 3.9	94.8 ± 3.9[a]

[1]Mean ± standard error.

[a]Significantly different from control ($P < 0.01$) - Dunnett's one-sided t-test.

in water consumption at concentrations up to 1000 ppm available chlorine (Cunningham, 1980).

As shown by the data presented in Tables 1 and 2, mink were more tolerant of sodium hypochlorite in their feed than in their drinking water. Consumption was reduced at 200 ppm NaOCl in the water while in the feed it was not reduced until a concentration of 3200 ppm NaOCl was fed. Perhaps, the NaOCl in the feed was masked by some of the other feed odors or flavors, making it more palatable to the mink. Another possibility is that the initial chlorine demand[3] of the water is met much more quickly than that of the feed. Since the amount of impurities and organic matter in the water is relatively low, the amount of chlorine required to meet this initial demand is low, thus free available chlorine for the mink to detect becomes available at lower concentrations of NaOCl. On the other hand, mink diets contain a considerable amount of organic matter and the amount of chlorine required to meet the initial demand is high and free available chlorine for the mink to detect may not become available until this demand is met.

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[3]"Chlorine demand" - the amount of initial chlorine that is used up by the water impurities and other organic materials. Chlorine is changed to inorganic chloride ions in these reactions.

AEROBIC PLATE COUNTS OF MINK FEED

Purpose

Mink ranchers who use fresh or frozen animal by-products in their feed are constantly exposing their animals to large numbers of bacteria which reside in these by-products. Many of these organisms, such as Staphylococci, Streptococci, and Salmonella, are potential disease producing organisms. Since NaOCl is effective against many of these microorganisms, it was thought that its addition to the feed would decrease the numbers of these microorganisms and decrease the rate of bacterial growth (spoilage) in the mink feed.

Materials and Methods

Five feed samples from the control diet and five from a diet containing 100 ppm supplemental NaOCl were placed in sterile petri dishes and left exposed to laboratory atmosphere at a temperature of 22 ^{o}C for 24 hours. Aerobic plate counts (Dunnigan, 1972; see Appendix D) were made on each sample (in duplicate) at 0, 3, 6, 12, and 24 hours to determine the amount of bacteria per gram of feed. Data were analyzed using a split-plot analysis with the feed samples serving as incomplete blocks.

Results

As shown in Table 3, both the control and NaOCl-treated feed had increasing bacterial counts over time. There was, however, no marked difference in trends between the two groups, nor were there any significant differences ($P < 0.05$) in the counts between the control feed and the NaOCl-treated feed at any of the five corresponding time periods. Sodium hypochlorite, when added to the feed at a concentration of 100 ppm did not significantly reduce the initial bacterial count of the mink feed, nor did it significantly reduce bacterial growth in the feed over a 24 hour time period.

Discussion

Although 100 ppm of supplemental sodium hypochlorite failed to significantly reduce bacterial growth in the feed, higher levels of NaOCl might be required to obtain positive results. Since 100 ppm NaOCl did not decrease the initial amount of bacteria in the diet when it was added, it is quite possible that most of the NaOCl was destroyed by its interaction with the organic matter in the feed, leaving no residual chlorine for the destruction of microorganisms. Coates (1977) found that all types of organic material interact with NaOCl causing a serious loss of disinfection activity. Yasukawa (1931) reported that 500 ppm of chlorine did not sterilize oysters in water containing *Escherichia coli* and *Eberthella typhosa*. He concluded that much

Table 3. Aerobic bacterial plate counts on control and NaOCl-treated feed over time.

	n	Time (Hours)				
		0	3	6	12	24
Control diet[1] (10^6 cts/gm)	5	0.61 ± 10.8	0.68 ± 10.8	1.26 ± 10.8	11.20 ± 10.8	176.80 ± 10.8
100 ppm supplemental NaOCl in feed (10^6 cts/gm)	5	0.54 ± 10.8	0.52 ± 10.8	0.91 ± 10.8	3.74 ± 10.8	184.30 ± 10.8

[1]Mean ± standard error.

of the free chlorine was used by organic matter and, thus, was not available for the destruction of bacteria in the oysters. Wabeck et al. (1967) reported that available chlorine is rapidly depleted in the presence of poultry meat and drumsticks. A constantly flowing 20 ppm chlorine solution did not reduce the total bacterial counts in poultry drumsticks. He concluded that chlorine concentrations must be metered into the system at a rate which overcomes the absorption rate by poultry.

Some chlorine treatments have been shown to be effective in the reduction of bacterial growth. After 10 days of storage at 38 ^{o}F, chicken fryers chilled for 2 hours in water containing 140 ppm chlorine contained 140,000 bacteria/gm while control fryers contained over 5,000,000 (Dawson et al., 1956). Mallman et al. (1959) found that up to 400 ppm chlorine in the chill water yielded birds with lower bacterial counts after seventeen days of storage.

These studies suggest that in order for sodium hypochlorite to be effective in the reduction of bacterial growth, chlorine levels must be high enough to ensure adequate residual chlorine to destroy bacteria after NaOCl has reacted with any organic matter present. Since mink will tolerate concentrations up to 1600 ppm supplemental NaOCl in their feed, additional studies should be conducted to determine whether concentrations higher than 100 ppm might reduce bacterial concentrations and growth in

conventional mink diets, and if this is the case whether these levels would have any adverse effects on growth and/or reproduction in the mink.

GROWTH AND REPRODUCTION OF MINK ADMINISTERED SODIUM HYPOCHLORITE

Purpose

Mink farmers use chemical disinfectants to sanitize cages, nest boxes, water cups, water lines, and feed equipment. Since chlorine disinfectants are inexpensive and effective, they are widely used for this purpose. The mink are constantly being exposed to these disinfectants. Recent research has shown that sodium hypochlorite - the major component of most chlorine disinfectants - has a growth stimulatory effect on rats and guinea pigs when administered at low concentrations (Cunningham, 1980).

This study was conducted to investigate any beneficial or adverse effects NaOCl might have on the growth and/or reproduction of mink.

Materials and Methods

The growth and reproduction study was started on July 2, 1980. Ninety standard dark mink were randomly allocated into six groups, each containing 12 females and 3 males. Littermates were placed on different treatment levels in order to avoid biasing the trial. Sodium hypochlorite was added either to the drinking water or the feed at the following concentrations.

Group I - No NaOCl added to the drinking water or the feed (control)

Group II - 25 ppm NaOCl in the drinking water

Group III - 50 ppm NaOCl in the drinking water

Group IV - 100 ppm NaOCl in the drinking water

Group V - 200 ppm NaOCl in the drinking water

Group VI - 100 ppm NaOCl in the feed -untreated drinking water

Each group (except Group VI) was fed a standard basal mink diet (see Appendix E). Group VI received the basal diet supplemented with 100 ppm NaOCl. The sodium hypochlorite was added to the mink feed daily, just prior to its being fed to the animals. Since NaOCl breaks down readily, especially in sunlight (Gelinas and Goulet, 1982), the mink water cups were emptied daily and refilled with fresh untreated or NaOCl-treated water twice daily (morning and evening).

The mink were housed in open-sided sheds and cared for according to standard mink ranch procedures (Travis and Schaible, 1960). From July 2, 1980 to February 2, 1981 the mink were housed in growing cages (61.5 cm length x 31.0 cm width x 38.5 cm height) with attached penthouse nest boxes (31.0 cm length x 23.0 cm width x 15.5 cm height). Thereafter the mink were placed in breeder cages (77.0 cm length x 61.5 cm width x 46.0 cm height) with attached outside nest boxes (38.5 cm length x 25.5 cm width x 25.5 cm height). At 10 weeks of age the mink were vaccinated for botulism, distemper and virus enteritis. Body weights were

recorded at the start of the experiment and at biweekly intervals from July 2 to September 24, and at monthly intervals thereafter, until the start of the breeding season (March 1).

During the breeding season, the female mink were mated with males within their respective treatment groups whenever possible. Matings were confirmed, by examining vaginal smears taken from the females immediately after copulation. The smears were examined for the presence of live, motile sperm. After the initial sperm checked mating the females were given an opportunity for a second mating either the day after the original mating or eight days later. At least two positive matings per female were obtained whenever possible. Beginning April 18, the nest boxes of female mink were checked daily for litters. The kits were counted, sexed and weighed at day 1, three weeks of age and six weeks of age.

Prior to the breeding season, blood samples were taken by heart puncture from four females in each group and submitted to the Michigan State University Veterinary Clinic for analysis of serum blood levels of Na^+, K^+, Cl^-, and total CO_2^-. Blood samples were also collected from all the mink by toe clips for red blood cell counts, white blood cell counts, hematocrit, and hemoglobin values. Blood smear were made to determine mean differential white cell counts. At the end of the trial, four female adult mink from Group I (control), Group V (200 ppm NaOCl in drinking water) and Group VI (100 ppm NaOCl in feed) were necropsied and their organs weighed.

Data were analyzed using one way analysis of variance. Treatment means were compared using Dunnett's two-sided t-test (Gill, 1978).

Results

Sodium hypochlorite when added to the drinking water or the feed, did not have a significant effect on mink body weight gains during the post weaning growing and furring periods (July 2 to November 20, Table 4) at the levels employed in this study. Male and female mink in all groups had increasing body weight gains over the course of the growing period. The female mink reached their full growth (maximum body weight) about 16 weeks after the start of the trial (24 weeks of age), whereas the males continued to gain weight (depending on the severity of the weather) up to 28 weeks after the trial began (36 weeks of age).

The NaOCl-treated water or feed had no effect on any of the hematologic parameters measured. No significant differences ($P < 0.05$) were detected between the control and treated groups in red blood cell counts, white blood cell counts, hematocrit or hemoglobin values (Table 5). Also, there was no effect on the differential white blood cell counts of mink in the various treatment groups (Table 6).

Serum blood levels of Cl^-, Na^+, K^+, and total CO_2^- were not affected by any of the treatments administered. There was a significant increase in the serum potassium (K^+) level of mink

Table 4. Mean initial body weights and weight gains from initial body weights at specified intervals of mink administered various concentrations of NaOCl in the drinking water or feed.

Group (concentration of supplemental NaOCl)	Sex	Initial wt 7-2-80	Weight gain (g)[1] 2 weeks 7-16-80	4 weeks 7-30-80	6 weeks 8-13-80	8 weeks 8-27-80	10 weeks 9-10-80	12 weeks 9-24-80	16 weeks 10-22-80	20 weeks 11-19-80	24 weeks 12-17-80	28 weeks 1-14-81
I (0 ppm; control)	M	533 ± 36.2	217 ± 22.0	457 ± 33.4	612 ± 43.3	760 ± 52.1	970 ± 47.4	915 ± 52.3	1195 ± 70.6	1260 ± 87.9	1153 ± 110.9	1322 ± 110.2
	F	462 ± 14.1	178 ± 9.1	313 ± 13.1	396 ± 19.1	435 ± 23.4	501 ± 26.1	481 ± 27.3	579 ± 26.5	581 ± 25.4	516 ± 25.9	551 ± 26.7
II (25 ppm in drinking water)	M	527 ± 36.2	255 ± 22.0	468 ± 33.4	657 ± 43.3	797 ± 52.1	993 ± 47.4	960 ± 52.3	1190 ± 70.6	1197 ± 87.9	1173 ± 110.9	1302 ± 110.2
	F	454 ± 14.1	181 ± 9.1	322 ± 13.1	417 ± 19.1	462 ± 23.4	515 ± 26.1	532 ± 27.3	605 ± 26.5	576 ± 24.3	515 ± 24.9	489 ± 25.5
III (50 ppm in drinking water	M	573 ± 36.2	200 ± 22.0	410 ± 33.4	583 ± 43.3	720 ± 52.1	923 ± 47.4	872 ± 52.3	1133 ± 70.6	1162 ± 87.9	1163 ± 110.9	1307 ± 110.2
	F	481 ± 14.1	164 ± 9.1	305 ± 13.1	417 ± 19.1	446 ± 23.4	499 ± 26.1	500 ± 27.3	574 ± 26.5	598 ± 24.3	554 ± 24.9	533 ± 25.5
IV (100 ppm in drinking water)	M	497 ± 36.2	251 ± 22.0	495 ± 33.4	693 ± 43.3	830 ± 52.1	1028 ± 47.4	973 ± 52.3	1270 ± 70.6	1422 ± 87.9	1368 ± 110.9	1382 ± 110.2
	F	455 ± 14.1	175 ± 9.1	318 ± 13.1	412 ± 19.1	451 ± 23.4	500 ± 26.1	491 ± 27.3	604 ± 26.5	585 ± 24.3	550 ± 24.9	549 ± 25.5
V (200 ppm in drinking water)	M	470 ± 36.2	202 ± 22.0	437 ± 33.4	663 ± 43.3	827 ± 52.1	1030 ± 47.4	1000 ± 52.3	1317 ± 70.6	1477 ± 87.9	1347 ± 110.9	1400 ± 134.9
	F	464 ± 14.1	179 ± 9.1	313 ± 13.1	421 ± 19.1	469 ± 23.4	513 ± 26.1	514 ± 27.3	603 ± 26.5	591 ± 24.3	526 ± 24.9	538 ± 25.5
VI (100 ppm in feed)	M	630 ± 36.2	200 ± 22.0	478 ± 33.4	705 ± 43.3	877 ± 52.1	1137 ± 47.4	1173 ± 52.3	1233 ± 70.6	1170 ± 87.9	1145 ± 110.9	1273 ± 110.2
	F	432 ± 14.1	165 ± 9.1	309 ± 13.1	398 ± 19.1	457 ± 23.4	498 ± 26.1	527 ± 27.3	638 ± 26.5	601 ± 24.3	562 ± 24.9	564 ± 25.5

[1] Mean ± standard error

Table 5. White blood cell counts, red blood cell counts, hematocrit, and hemoglobin values of mink administered various concentrations of NaOCl in the drinking water or feed.

Group (concentration of supplemental NaOCl)	Sex	n	White blood cells ($10^3/mm^3$)	Red blood cells ($10^6/mm^3$)	Hemoglobin (g%)	Hematocrit (%)
I						
(0 ppm, Control)	M	3	16832 ± 3081[1]	10.17 ± 0.41	24.2 ± 0.82	57.3 ± 1.03
	F	11	25597 ± 2234	9.15 ± 0.22	21.2 ± 0.51	52.2 ± 1.03
II						
(25 ppm in	M	3	13719 ± 3081	9.61 ± 0.41	22.9 ± 0.82	57.0 ± 1.03
drinking water)	F	12	26400 ± 2139	8.91 ± 0.21	20.3 ± 0.49	51.7 ± 0.99
III						
(50 ppm in	M	3	13333 ± 3081	10.68 ± 0.41	23.7 ± 0.82	57.8 ± 1.03
drinking water)	F	11	18809 ± 2234	9.44 ± 0.22	21.6 ± 0.51	54.8 ± 1.03
IV						
(100 ppm in	M	3	17271 ± 3081	9.00 ± 0.41	22.5 ± 0.82	56.7 ± 1.03
drinking water)	F	12	23139 ± 2139	9.22 ± 0.21	21.7 ± 0.49	53.4 ± 0.99
V						
(200 ppm in	M	2	21300 ± 3773	10.06 ± 0.50	23.1 ± 1.00	56.9 ± 1.26
drinking water)	F	11	21947 ± 2234	9.34 ± 0.22	21.3 ± 0.51	54.3 ± 1.03
VI						
(100 ppm in feed)	M	3	12556 ± 3081	9.73 ± 0.41	22.8 ± 0.82	55.9 ± 1.03
	F	12	23286 ± 2139	9.46 ± 0.21	22.0 ± 0.49	54.1 ± 0.99

[1]Mean ± standard error.

Table 6. Mean differential white blood cell counts of mink administered various concentrations of NaOCl in the drinking water or feed.

Group (Concentration of supplemental NaOCl)	Sex	n	Cell type (%)[1] Basophil	Eosinophil	Band neutrophil	Mature neutrophil	Lymphocyte	Monocyte
I (0 ppm Control)	M	3	0.7 ± 0.3	1.3 ± 0.9	2.3 ± 0.9	46.0 ± 5.6	44.7 ± 6.5	5.0 ± 1.8
	F	11	0.2 ± 0.2	2.5 ± 0.4	3.7 ± 0.8	63.8 ± 2.5	25.1 ± 2.7	4.7 ± 0.7
II (25 ppm in drinking water)	M	3	0.0 ± 0.3	0.7 ± 0.9	2.0 ± 0.9	52.7 ± 5.6	41.3 ± 6.5	3.7 ± 1.8
	F	12	0.4 ± 0.2	1.6 ± 0.4	4.6 ± 0.8	60.4 ± 2.4	28.2 ± 2.6	4.9 ± 0.7
III (50 ppm in drinking water)	M	3	0.0 ± 0.3	3.0 ± 0.9	4.0 ± 0.9	53.7 ± 5.6	33.7 ± 6.5	5.7 ± 1.8
	F	11	0.3 ± 0.2	1.2 ± 0.4	4.3 ± 0.8	62.1 ± 2.5	26.3 ± 2.7	5.9 ± 0.7
IV (100 ppm in drinking water)	M	3	1.0 ± 0.3	1.0 ± 0.9	1.7 ± 0.9	48.0 ± 5.6	44.7 ± 6.5	3.7 ± 1.8
	F	12	0.3 ± 0.2	1.6 ± 0.4	3.2 ± 0.8	63.2 ± 2.4	26.8 ± 2.6	5.1 ± 0.7
V (200 ppm in drinking water)	M	2	0.0 ± 0.4	1.0 ± 1.1	2.5 ± 1.1	50.0 ± 6.8	43.5 ± 7.9	3.0 ± 2.3
	F	11	0.1 ± 0.2	1.1 ± 0.4	4.5 ± 0.8	59.6 ± 2.5	31.0 ± 2.7	3.7 ± 0.7
VI (100 ppm in feed)	M	3	0.0 ± 0.3	3.3 ± 0.9	3.7 ± 0.9	45.0 ± 5.6	46.0 ± 6.5	2.0 ± 1.8
	F	12	0.3 ± 0.2	1.2 ± 0.4	4.6 ± 0.8	61.9 ± 2.4	28.3 ± 2.6	3.8 ± 0.7

[1]Mean ± standard error.

receiving 50 ppm NaOCl, but this trend was not verified in any of the other NaOCl-treated groups, which were comparable to the control group (Table 7).

Organ weights taken at the conclusion of the experiment from the female mink in groups I, V, and VI, showed no effect of NaOCl on any of the organs measured (Table 8). Upon gross examination, all organs appeared normal.

The reproductive performance of females in the various treatment groups was comparable to that of the control group. There were no significant differences in conception rate, gestation period, or average number of live kits at birth per female whelped between the control and various NaOCl-treated groups (Table 9). Although, as shown in Table 9, there was no significant difference in the average number of kits at 3 weeks between the control group and any of the NaOCl-treated groups, greater kit mortality between birth and three weeks was noted among the higher NaOCl-treated groups. There was no kit mortality in any of the groups between three and six weeks of age.

As shown in Table 10, there was no correlation between the level of NaOCl administered and kit body weights. Kit body weights at day 1, 3 weeks, and 6 weeks of age were generally comparable to those of the control group. The birth weight of kits from females in Group VI were significantly less than those of the control kits, however, at 6 weeks of age the male kits in this group weighed more than the controls. Also, the female kits

Table 7. Mean serum blood level values of Cl^-, Na^+, K^+, and total CO_2^- of mink given various concentrations of NaOCl in the drinking water or feed.

Group (concentration of supplemental NaOCl)	Serum Concentration (mEq/L)[1]			
	Cl^-	Na^+	K^+	Total CO_2^-
I (0 ppm; Control)	109.0 ± 0.89	147.0 ± 1.86	4.03 ± 0.17	25.5 ± 3.25
II (25 ppm in drinking water)	109.5 ± 0.89	147.3 ± 1.86	4.28 ± 0.17	23.7 ± 2.65
III (50 ppm in drinking water)	109.5 ± 0.89	150.8 ± 1.86	4.83 ± 0.17[a]	23.7 ± 2.65
IV (100 ppm in drinking water)	111.5 ± 0.89	148.3 ± 1.86	4.15 ± 0.17	23.0 ± 2.65
V (200 ppm in drinking water)	109.3 ± 0.89	152.5 ± 1.86	4.20 ± 0.17	23.0 ± 2.65
VI (100 ppm in feed)	109.3 ± 0.89	150.3 ± 1.86	4.05 ± 0.17	24.5 ± 2.30

[1]Mean ± standard error.

[a]Significantly different from control ($P < 0.01$) by Dunnett's t-test.

Table 8. Organ weights of female mink from the control group, and groups treated with 200 ppm NaOCl in the water or 100 ppm NaOCl in the feed.

Treatment	n	Organ weight[a,b]						
		Spleen	Kidney	Lung	Heart	Brain	Liver	Body weight (g)
Control	4	0.41 ± 0.06[c]	0.59 ± 0.04	0.83 ± 0.07	0.58 ± 0.02	0.92 ± 0.10	4.31 ± 0.33	760.5 ± 47.1
200 ppm NaOCl in drinking water	4	0.43 ± 0.05	0.63 ± 0.04	0.85 ± 0.07	0.60 ± 0.02	1.05 ± 0.10	3.96 ± 0.33	861.8 ± 47.1
100 ppm NaOCl in feed	4	0.39 ± 0.05	0.54 ± 0.04	0.80 ± 0.08[c]	0.59 ± 0.02	1.01 ± 0.10	3.63 ± 0.33	820.5 ± 47.1

[a]Expressed as a % of body weight.

[b]Mean ± standard error.

[c]n=3

Table 9. Reproductive performance of female mink administered various concentrations of NaOCl in the drinking water or feed and survivability of their kits.

Group (concentration of supplemental NaOCl)	No. females whelped/ no. mated	Gestation (days)	Avg. no. kits/female whelped			Kit mortality (%)	
			At Day 1	At 3 wks.	At 6 wks.	Birth to 3 wks.	3 wks. to 6 wks.
I (0 ppm; Control)	8/11	47.0 ± 1.4[1]	6.0 ± 0.69	5.6 ± 0.68	5.6 ± 0.73	6.3	0
II (25 ppm in drinking water)	12/12	47.5 ± 1.2	5.3 ± 0.56	5.1 ± 0.56	5.1 ± 0.56	3.2	0
III (50 ppm in drinking water)	8/11	48.5 ± 1.4	6.0 ± 0.69	5.0 ± 0.73	5.0 ± 0.73	27.0	0
IV (100 ppm in drinking water)	10/12	47.0 ± 1.3	5.3 ± 0.62	3.9 ± 0.61	3.9 ± 0.61	26.4	0
V (200 ppm in drinking water)	8/10	46.1 ± 1.4	5.8 ± 0.69	5.0 ± 0.68	5.0 ± 0.68	13.0	0
VI (100 ppm in feed)	10/12	48.3 ± 1.3	5.6 ± 0.62	4.4 ± 0.61	4.4 ± 0.61	19.6	0

[1]Mean ± standard error.

Table 10. Mean body weight at Day 1, 3 weeks, and 6 weeks of kits whelped and nursed by females administered various concentrations of NaOCl in the drinking water or feed.

Group (concentration of supplemental NaOCl)	Kit body weight (g)[1]			
			6 wks.	
	Day 1	3 wks	Males	Females
I (0 ppm; Control)	8.9 ± 0.22	103 ± 2.1	276 ± 11.5	272 ± 10.1
II (25 ppm in drinking water)	8.6 ± 0.19	102 ± 1.8	286 ± 9.8	248 ± 7.7
III (50 ppm in drinking water)	9.2 ± 0.22	90 ± 2.4[a]	261 ± 11.9	224 ± 10.9[a]
IV (100 ppm in drinking water)	8.5 ± 0.23	108 ± 2.3	279 ± 12.6	260 ± 9.9
V (200 ppm in drinking water)	8.5 ± 0.23	105 ± 2.2	301 ± 10.7	252 ± 10.6
VI (100 ppm in feed)	7.9 ± 0.21[a]	103 ± 2.1	313 ± 10.2	262 ± 10.9

[1]Mean ± standard error.

[a]Significantly less than control ($P < 0.01$) - Dunnett's one-sided t-test.

from females in Group III weighed significantly less than the control female kits at 6 weeks of age.

Discussion

The levels of sodium hypochlorite chosen for this study were derived from the water consumption data reported earlier. Since water consumption was reduced significantly at 200 ppm supplemental NaOCl, this concentration was chosen as the highest level of administration. This was done to avoid any effects of dehydration confounding the effects of NaOCl. Although mink can tolerate higher levels of NaOCl in the feed than the 100 ppm supplemental NaOCl used in this study, this level was used so that comparisons could be made between the administration of NaOCl in the feed and in the drinking water.

The addition of NaOCl at concentrations up to 200 ppm to mink drinking water or feed had no effect (stimulating or depressing) on body weight gains during the growing period. These results do not support the findings of Cunningham (1980) who reported increased body weight gains in rats and guinea pigs administered supplemental NaOCl in their water at concentrations up to 80 ppm available chlorine with a maximum increase at 40 ppm. Hulan and Proudfoot (1982) reported no effect on body weight gains of broiler chicks administered up to 150 ppm available chlorine. At concentrations above 300 ppm they found poorer feed conversion (decreased weight gains) and increased

mortality. These adverse effects noticed at the higher concentrations of chlorine in broilers may be due to dehydration effects and not the effects of the sodium hypochlorite itself, since there was a decrease in water consumption at concentrations above 300 ppm. Since broilers consume a dry ration, any decrease in water consumption may lead to poorer feed conversion and increased mortality. None of these adverse effects were noticed with the mink, but the NaOCl levels administered to the mink were not nearly as high as those given to the chickens. Although a decrease in water consumption was reported at the 200 ppm concentration of NaOCl used in this trial, this did not produce any adverse effects in the mink. This could be due to the fact that the mink's feed is approximately 60% water which may supply sufficient water to meet its requirement.

Cunningham (1980) suggested that the means by which NaOCl might improve weight gain is that it may lessen the microbiological flora inhabiting the digestive tract and competing with the animal for food. One possible explanation why this increased weight gain was not seen in the mink is that the mink have a very short digestive tract and continually consume food to meet their energy needs. Any NaOCl that they ingest immediately interacts with the organic matter (feed) in their digestive tract and renders it ineffective for destroying the bacteria in the intestinal tract.

NaOCl at concentrations up to 200 ppm produced no significant effects on any of the hematologic parameters

measured. Sodium hypochlorite is probably a less potent oxidant stressor to the blood than chlorite (ClO_2^-), since chlorite at concentrations of 100 ppm and above has been shown to decrease red blood cell counts, hemoglobin concentration and packed cell volume in the rat at 30 and 60 days exposure (Heffernan et al., 1979). Very similar results were reported in the mouse by Moore and Calabrese (1980).

Sodium hypochlorite forms chloride ions when it interacts with inorganic ions and organic material in water or feed. Since the interaction of Cl^-, K^+, Na^+, and total CO_2^- determines the pH of the blood, it is possible that excess chloride ions could exert a profound effect on the acid-base balance of the blood. The data presented in Table 7 indicates that the addition of NaOCl, in low concentrations to the water or feed of mink, had no adverse effects on this interaction. The serum blood levels of Cl^-, K^+, Na^+, and total CO_2^- for animals treated with NaOCl were within the normal range for mink (Anonymous, 1980).

The most common signs of chlorine toxicosis in rats are reduced body weight gain and enlarged liver, kidney and heart (Cunningham et al., 1977; Cunningham and Lawrence, 1978). The amount of chlorine required to produce toxicity was 2,000 to 10,000 mg/kg in the diet. Hufran and Proudfoot (1982) also reported decreased weight gains in broiler chicks administered concentrations above 600 ppm chlorine in their water, but lower kidney, heart and testes weight. Such changes in organ weights

were not observed in this trial, but the levels of sodium hypochlorite administered were not as high.

Chlorite (ClO_2^-), when administered at 100 ppm in the water, has been shown to be capable of reducing the conception rate of A/J mice (Moore et al., 1980). This effect was not seen with the NaOCl in this study. There was no significant differences in the reproductive performances of female mink between the control and the various treatment levels of NaOCl. Greater kit mortality was noticed between birth and three weeks of age among the higher NaOCl-treated groups, suggesting that young kits may be more susceptible to NaOCl than older mink. Examination of the data however, revealed that this higher mortality rate was accounted for by a few females in each of the groups that lost a large portion of their litters. This suggests that they were either very poor mothers or possibly did not have enough mammary development to support all the kits.

Although there were a few significant differences in kit body weights at day 1, 3 weeks, and 6 weeks of age between the control and the NaOCl-treated mink, no trend between the level of NaOCl administered and kit body weights could be established. These results do not support the findings of Moore et al., (1980) who reported a reduced growth rate in mouse pups whose dams were administered 100 ppm chlorite (ClO_2^-) in their drinking water. These findings suggest that NaOCl is not as toxic as chlorite or possibly acts in a different manner.

CONCLUSIONS

1. Mink are less tolerant of sodium hypochlorite in their drinking water than in their feed. Water consumption was reduced at 200 ppm NaOCl in the drinking water. Feed consumption was reduced at 3200 ppm NaOCl in the food.

2. One hundred ppm NaOCl added to the feed did not significantly reduce the amount of bacteria in the feed nor did it significantly slow bacterial growth (spoilage).

3. Sodium hypochlorite added to the water at concentrations up to 200 ppm and to the feed at 100 ppm showed no effects on mink growth or reproduction.

APPENDICES

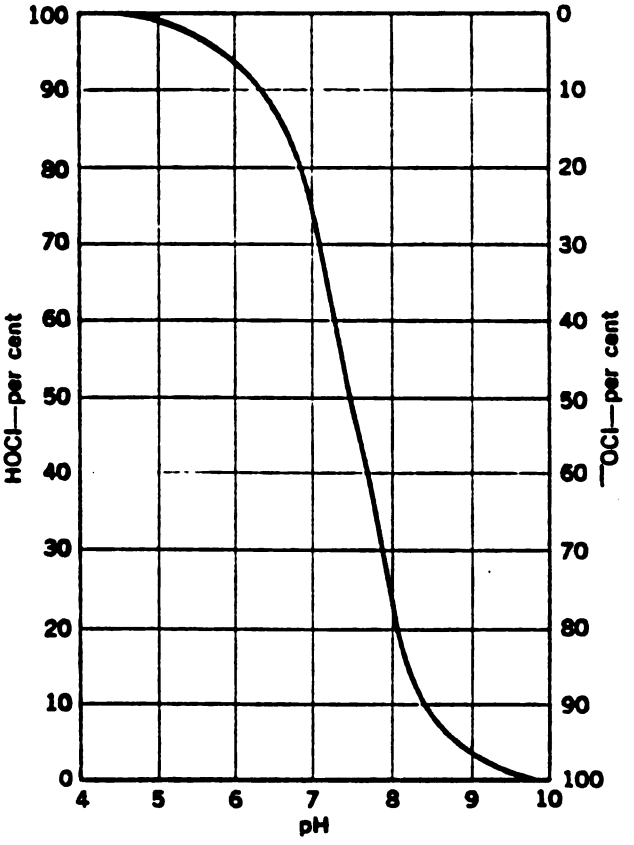
Appendix A. The concentrations of chlorine disinfectants (including NaOCl) commonly used in different industries.

Industry	Use	Concentration of available chlorine (ppm)	Reference
Swimming Pool Water	Chlorination of water for disinfection	1 to 2	Robinton et al., 1957
Swimming Pool Water	Algae control	0.2	Lackey et al., 1964
Spa's and Hot Tubs	Chlorination of water for disinfection	1 to 3	Anonymous, 1980
Dairy	Sanitization of equipment and utensils	50 to 100	Anonymous, 1965
Food Plants	In-plant chlorination	10 to 25	Somers, 1951

Appendix B. Biocidal effect of free available chlorine on various organisms (After Drychaula, 1983).

Organism	pH	Temp. (°C)	Exposure Time	ppm Av. Cl_2	Biocidal Results
ALGAE					
Chlorella variegata	7.8	22	---	2.0	Growth controlled
Gomphonema parvulum	8.2	22	---	2.0	Growth controlled
Microcystis aeruginosa	8.2	22	---	2.0	Growth controlled
BACTERIA					
Achromobacter metalcaligenes	6.0	21	15 seconds	5.0	100%
Bacillus anthracis	7.2	22	120 minutes	2.3-2.4	100%
B. globigii	7.2	22	120 minutes	2.5-2.6	99.99%
Clostridium botulinum toxin type A	7.0	25	30 seconds	0.5	100%
Escherichia coli	7.0	20-25	1 minute	0.055	100%
E. typhosa	8.5	20-25	1 minute	0.1-0.29	100%
Mycobacterium tuberculosis	8.4	50-60	30 seconds	50	100%
Pseudomonas fluorescens LM	6.0	21	15 seconds	5.0	100%
Shigella dysenteriae	7.0	20-50	3 minutes	0.046-0.055	100%
Staphylococcus aureus	7.2	25	30 seconds	0.8	100%
Streptococcus faecalis	7.5	20-50	2 minutes	0.5	100%
All vegetative bacteria	9.0	25	30 seconds	0.2	100%
BACTERIOPHAGE					
S. cremoris phage strain 114F	6.9-8.2	25	15 seconds	25	100%
FISH					
Carassius auratus	7.9	Room	96 hours	1.0	Killed
Daphnia magna	7.9	Room	72 hours	0.5	Killed
FROGS					
Rana pipiens	8.3	21	4 days	10	100%
FUNGI					
Aspergillus niger	10-11	20	30-60 minutes	100	100%
Rhodotorula flava	10-11	20	5 minutes	100	100%
NEMATODES					
C. quadrilabiatus	6.6-7.2	25	30 minutes	95-100	93%
D. nudicapitatus	6.6-7.2	25	30 minutes	95-100	97%
PLANTS					
Cabomba caroliniana	6.3-7.7	Room	4 days	5	100%
Elodea canadensis	6.3-7.7	Room	4 days	5	100%
PROTOZOA					
Endamoeba histolytica cysts	7.0	25	150 minutes	0.08-0.12	99-100%
VIRUSES					
Purified adenovirus 3	8.8-9.0	25	40-50 seconds	0.2	99.8%
Purified Coxsackie A_2	6.9-7.1	27-29	3 minutes	0.92-1.0	99.6%
Purified Coxsackie B_1	7.0	25	2 minutes	0.31-0.40	99.9%
Purified Coxsackie B_5	7.0	25-28	1 minute	0.21-0.30	99.9%
Infectious hepatitis	6.7-6.8	Room	30 minutes	3.25	Protected all 12 volunteers
Purified poliovirus (Mahoney)	7.0	25-28	3 minutes	0.21-0.30	99.9%
Purified poliovirus (Lansen)	7.4-7.9	19-25	10 minutes	1.0-0.5	Protected all 164 inoculated mice
Purified poliovirus III (Sankett)	7.0	25-28	2 minutes	0.11-0.2	99.9%
Purified Theiler's	6.5-7.0	25-27	5 minutes	4-6	99.9%

Appendix C. Relationship between HOCl, $^{-}$OCl, and pH. (After Baker, 1959).



Appendix D. Procedure for aerobic plate counts of mink feed.

1. Ten grams of mink food was weighed aseptically into a sterile jar of 200 ml capacity. 90 ml of sterile water was then added providing a 10^{-1} dilution.
2. The food homogenate was blended for 2 minutes at 8000 RPM.
3. Using separate sterile pipettes, decimal dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} of the food homogenate were prepared.
4. Decimal dilutions were prepared by transferring 1 ml of the previous dilution to 9 ml of the diluent (sterile distilled water). All dilutions were shaken 25 times in a 1-foot arc within 7 seconds.
5. Each decimal dilution was agitated to suspend any material that may have settled out and 1 ml of each dilution was then pipetted into each of appropriately marked duplicate petri dishes.
6. The plates were filled with 12 to 15 ml of Brain Heart Infusion Agar (cooled at 45 ^{o}C) within 15 minutes of the time of the original dilution.
7. The samples dilutions and agar medium were mixed immediately by rotating the plates on a flat surface.
8. The agar was allowed to solidify, the petri dishes inverted, and incubated promptly at 35 ^{o}C for 48 hours.

9. Following incubation, all colonies on plates containing 30-300 colonies were counted, using a colony counter and tally register. Results were recorded per dilution counted.
10. The counts obtained were averaged and reported as counts per gram of feed.

Appendix E. Composition of basal mink diet.

Ingredients	Percentage of diet
Mink cereal[1]	20
Whole chicken	24
Ocean fish scraps[2]	15
Beef tripe	8
Beef lungs	4
Beef trimmings	4
Beef liver	4
Water	21

[1]XK-40 Mink Food. XK Mink Food, Inc. Subsidiary of United Feeds, Inc., Theinsville, WI.

[2]Cod, haddock and flounder mix, G.M.F. Corp., Glouchester, MA.

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