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Subject: Comments Regarding NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate (NTP TR 546)

Dear Dr. Shane:

Please accept the attached comments on the above-referenced Technical Report (CAS No. 7789-12-0), for consideration during the May 16–17, 2007 peer review. These comments were prepared by Exponent, Inc. at the request of Tierra Solutions, Inc.

The findings of the NTP Sodium Dichromate Dihydrate drinking water study are of great significance for regulatory assessment of the human health risk associated with ingested hexavalent chromium [Cr(VI)]. Our comments present questions that we have identified as important to understanding these results, followed by supplemental information and analysis of the relevant studies.

We look forward to the review board's consideration and discussion of our comments.

Best regards,

Deborah Proctor
Principal Health Scientist

Exponent®

Comments Regarding NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate (CAS No 7789-12-0), for May 16–17, 2007 Peer Review

The findings of the NTP Sodium Dichromate Dihydrate drinking water study are of great significance for regulatory assessment of the human health risk associated with ingested hexavalent chromium [Cr(VI)]. Therefore, it is important to conduct a thorough and balanced analysis of these findings.

On the behalf of Tierra Solutions, Inc., Exponent has identified several questions, offered below, that are important to discuss for a thorough understanding of the NTP study results. In addition, we have provided supplemental information and analysis of the findings for consideration in addressing these questions.

Questions

1. NTP study protocol requires that the highest dose administered in a study not exceed the maximum tolerated dose (MTD) for the chemical. Based on effects observed in subchronic studies, NTP reduced the highest dose of the chronic study to 516 mg/L. Given that more than 10% reduction in body weight was observed at the highest dose (516 mg/L), as well as other adverse effects, was the MTD of sodium dichromate dehydrate exceeded at the 516 mg/L dose in this study?

2. In this study, reductions in body weight and water consumption were observed, particularly in the latter half of the study. These effects indicate that these animals may have been dehydrated. If so, could dehydration, and the associated decrease in salivation or other related physiological alterations, be a factor in the development of oral mucosal tumors in rats?

3. NTP cites reviews of epidemiological studies—such as Costa (1997), Hanslian et al. (1967), which is a descriptive study of oral papillomas among Czechoslovakian chromium platers, and the Zhang and Li (1987) ecologic study of Chinese villagers in support of an association between Cr(VI) and cancers outside the respiratory tract in humans. However, we reviewed all epidemiological studies (e.g., cohort, case-control design) that we could locate in the peer-reviewed literature, which examined the association between Cr(VI) and oral cancer among Cr(VI)-exposed workers, and observed no evidence of an association between Cr(VI) exposure and the risk of oral cancers. In short, none of the 17 identified studies reported a

statistically significant increase in oral mucosa cancers (Table 1). Similarly, we reviewed the epidemiology literature for cancers of the small intestine among Cr(VI)-exposed workers, and none of the nine studies we located, which evaluated small intestinal or intestinal cancers, had a statistically significant increase (Table 2). We request that this disparity be given additional attention from the review panel.

4. Non-neoplastic lesions (e.g., hyperplasia, ulcers) were not observed in the oral mucosa of rats (page 83 of the NTP report), although oral mucosa carcinomas were. Direct toxicity from Cr(VI) exposure in the oral mucosa epithelium is expected to cause tissue damage consistent with a site-of-contact toxicity and carcinogenicity mode-of-action. Is there an explanation for this lack of non-neoplastic lesions in the rat oral mucosa?

5. Oral mucosa tumors were reported only in rats, whereas intestinal tumors were observed only in mice. However, in mice tissues that were likely exposed to higher levels of Cr(VI), such as the forestomach and stomach, did not develop tumors. How can these disparate patterns of tumor development be explained on both an intra-species and an inter-species basis?

6. Histiocytic infiltration was observed in the liver, in the intestine, and in pancreatic and mesenteric lymph nodes, suggesting chronic irritation of the intestinal epithelium. Based on these findings, NTP concluded that the intestinal epithelial hyperplasia is a pre-neoplastic lesion related to the intestinal tumors (page 74 of the NTP report). As hyperplasia is associated with chronic tissue irritation, might these observations indicate a non-genotoxic mode-of-action for the small intestine tumors observed in mice?

7. The concentrations of Cr(VI) administered to the rodents far exceed Cr(VI) exposures that humans are expected to incur from drinking water and/or in the environment. Recognizing that the reductive capacity of the rodent stomach is saturable, is the likely mode-of-action for carcinogenicity observed in this study at high concentration exposures in rodents relevant for humans and what factors should be considered in extrapolating the study findings for assessing risk in humans with low-level exposure?

8. Differences in kinetics between rats and mice are evident in the tissue data presented in Appendix J of the NTP study. We specifically note that the levels of Cr in mouse liver, by dose group, are much higher than in rats, and that Cr levels in feces, adjusted by mean body weight, in the rat are higher than in mice. Does this suggest that the reductive capacity of the rat stomach is greater than that of the mouse, and that small-intestine tumors observed in the mouse are potentially related to this interspecies kinetic difference?

9. Many differences exist in the physiology and anatomy of the rat and mouse gastrointestinal tracts, with even greater differences in humans. One such difference of particular importance is basal rate of gastric acid secretion, which is approximately 1,200 times greater in the rat compared to the mouse (Friis-Hansen et al. 1998; Runfola et al. 2003; Wang et al. 2003). The human

basal gastric acid secretion rate is approximately 8-times higher than that of the rat (Friis-Hansen et al. 1998). Are these kinetic differences important in understanding the interspecies variability observed in the study?

10. The lack of tumors outside the gastrointestinal (GI) system, even at the doses that likely overwhelmed the reductive capacity of the stomach of these animals, is an important observation. The NTP report notes that, at the concentrations administered, Cr(VI) overwhelmed the reductive capacity of the stomach and circulated systemically. However, even at the very high level exposures to Cr(VI) in the NTP study, no increase in tumors was observed in distant tissues. Is there an explanation for this observation?

Supplemental information to consider in addressing these questions is provided below.

Comparison of Cr(VI) Concentrations Administered in the NTP Study with Potential Human Exposure Levels

The Cr(VI) exposures in the current NTP study far exceed expected human exposures. Extrapolation of these findings to low-dose human exposures is made more difficult by evidence that these exposures seem to have overwhelmed the reductive capacity of the rodent stomach; therefore, extrapolation from high doses in rodents to low doses in humans is likely non-linear. While recognizing that rodent cancer bioassays are typically performed at doses that far exceed human exposures, the following comparisons are relevant because of the well-recognized biological mechanisms that detoxify Cr(VI)—through reduction—when doses do not overwhelm the body's natural defenses. We request that the review panel compare the concentrations administered in the NTP study to following realistic human exposures.

- **US Drinking Water Exposures (per USGS and EPA):** An extensive data set of potential drinking water data developed by the U.S. Geological Survey (USGS), EPA and the State of Texas of Cr(VI) and total chromium in ambient groundwater wells was evaluated with a goal of understanding possible Cr(VI) exposures via drinking water. There are approximately 25,600 samples in this database analyzed for total chromium and 4,600 analyzed for Cr(VI) from locations across the United States. Cr(VI) was not detected in 48% of the samples in which it was analyzed. The geometric mean and 95th percentile Cr(VI) concentrations were 1.5 and 16 μg/L, respectively, and those for total chromium were 5.8 and 50 μg/L, respectively. Thus, the lowest dose group in the NTP study consumed Cr(VI) at concentrations more than 300 times higher than the 95th percentile of Cr(VI) concentrations to which people in the US are likely to be exposed.

- **California Drinking Water Exposures:** Cr(VI) is detectable in approximately one-third of 7,000 monitored drinking-water sources in California. Of the sources with measured levels of Cr(VI), 86% had peak concentrations less than 10 μg/L, and 65% had peak detections less than 5 μg/L. These concentrations are 500 and 1,000 times lower than the lowest

concentration tested in the NTP study, and 18,000 and 36,000 times lower than the highest concentrations tested. Compared to the lowest dose that resulted in a statistically significant increase in cancer in mice in the NTP study, these California drinking-water concentrations are 3,000 and 6,000 times lower.

- **Federal and State MCLs:** Federal and State maximum contaminant levels (MCLs) for total chromium are 100 and 50 μg/L, respectively. It is reasonable to assume that the MCLs provide a practical limit to the levels of Cr(VI) that could potentially be in drinking water supplies. The MCLs are 50 and 100 times lower than the lower bound of the administered concentrations in the NTP study, and 1,800 and 3,600 times lower than the highest concentrations tested.

- **Monitoring Well (Not Drinking Water) in Hinkley, California:** NTP cited a non-peer-reviewed source (Pellegrin and Booker 2000) regarding the concentration of Cr(VI) in a monitoring well in Hinkley, California. Regardless of whether this is an accurate citation, this information is not representative of drinking water concentrations in California or elsewhere. Further, the citation is inconsistent with the ATSDR (2000a) Public Health Assessment (PHA) for Hinkley. The ATSDR PHA states that one drinking-water well in Hinkley was found to contain Cr(VI) at a level of 20 μg/L.

Comparison of High-End Exposures to the MTD

According to NCI and IARC guidelines, the MTD is the dose that "causes no more than a 10% weight decrement as compared to the appropriate control group" (Bucher et al. 1996; Bucher 2000, 2002; Chhabra et al. 1990; Matsumoto et al. 2006; Morrow et al. 1996). There was a greater than 10% decrement in body weight for male rats, female rats, and female mice in the highest dose groups (Figure 1). While decreased body weight was likely related to poor water palatability, the effects of dehydration due to decreased water consumption may have exerted an effect on tumor development that was not related to Cr(VI). The highest dose groups of male and female mice decreased their water consumption by more than 30%, and the highest dose groups of male and female rats decreased their water consumption by more than 20% (Figure 2). Although it is unclear if related, salivary gland atrophy occurred in female rats at exposures of 172 and 516 mg/L. NTP (1994) indicates that signs of dehydration include decreased mean body weight, increased alanine aminotransferase (ALT) activities, and increased erythrocyte counts, all of which occurred with statistical significance in high-dose rats and mice. Unfortunately, other recognized indications of dehydration, including urinary output and other urinary parameters, were not measured, and hematology parameters were measured in only the first year of the study.

Dehydration is related to decreased salivary secretion in the rat (Ito et al. 2001). Several studies have shown that saliva has a protective role against cancer development in the oral cavity of animals (Dayan et al. 1997; Kaplan et al. 2002; Vered et al. 2003). Thus, decreased water consumption, even if due only to poor palatability, is likely a factor in the development of oral

mucosa tumors in the high-dose rats. Considering that the tumors appeared in the apparent absence of nonneoplastic lesions of the oral mucosa, it is questionable whether the direct mutagenic activity of Cr(VI), in the development of these tumors, is a reasonable mode-of-action.

Risk of Oral Cavity Tumors and Small Intestine Tumors in Cr(VI)-Exposed Workers

Human epidemiologic data do not support a position that oral exposures to Cr(VI) pose a cancer hazard in the oral cavity or small intestine. Exponent, Inc. searched the published literature to identify epidemiologic studies of occupational cohorts exposed to Cr(VI) that reported risk of cancers of the oral cavity and small intestine. Given that Cr(VI) has been studied extensively as a human respiratory carcinogen, a wealth of epidemiologic data exists. The objective of this review was to determine whether exposure to Cr(VI) is associated with an increased risk of oral and intestinal cancers.

The literature review identified 17 epidemiologic studies that considered oral cancers as one of many cancer outcomes examined with respect to Cr(VI) exposure. Measures of effect (e.g., standardized mortality ratio [SMR], Relative Risk [RR]) and variance (usually the 95% confidence interval) from each study were extracted from the publication. Study-specific relative risk estimates for oral cancers ranged between 0.32 and 2.5. Six studies reported effect estimates >1.0, and eleven studies reported effect estimates <1.0. However, none of the findings were statistically significant. This analysis of the published epidemiologic studies of occupational cohorts exposed to Cr(VI) shows a lack of association between Cr(VI) exposure and risk of oral cancers. These findings are summarized in Table 1.

Similarly, epidemiologic studies that considered intestinal cancer as one of several cancer outcomes were located and reviewed. Only three epidemiologic studies of workers exposed to Cr(VI) evaluated small intestine cancer risk. An additional six studies reported combined risk estimates for cancers of the small intestine, colon, and rectum. Of the three studies that evaluated cancer of the small intestine, Rafnsson et al. (1997) reported a SMR of 4.23 (95% CI = 0.85, 12.35) based on three cases, Iaia et al. (2006) reported a SMR of 2.04 (95% CI = 0.05, 11.4) based on one case, and Sorahan et al. (1994) reported a SMR = 0.55 (95% CI = 0.01, 3.04), also based on one case. SMRs for cancers of the intestine (including colon and rectum) ranged from 0.33 to 1.66, none of which were statistically significant (Table 2). While the body of data available to assess the risk of small intestine cancer among Cr(VI)-exposed workers is limited, the lack of evidence of an increased risk of small intestine cancer is noteworthy. Small intestine cancer is relatively rare—thus, even slight elevations would likely be evident—and dozens of studies have reported SMRs for stomach cancer and lung cancer. However, these same studies do not identify small-intestine cancer as a cancer outcome associated with Cr(VI) exposure.

Review of Hanslian et al. (1967) Study of Czechoslovakian Chrome Platers

NTP cites the Hanslian et al. (1967) study of Czechoslovakian chrome platers, as cited in ATSDR (2000b), as evidence of oral cancers associated with Cr(VI) exposure. This paper is a descriptive study, reporting papillomas of the mouth and larynx, based on examinations of workers at several chrome plating plants. Hanslian et al. (1967) is not an association study and should not be used to conclude that Cr(VI) exposure causes oral cavity papillomas. The authors did not use an analytical epidemiologic study design, and thus did not examine associations between Cr(VI) exposure and oral cancer risk. The study is limited to reports of a specific group of workers, and therefore, it is not possible to make inferences about the risk of oral cancers from workplace exposure to Cr(VI). The authors provide proportions of papillomas occurring in other occupations and suggest that there are fewer oral papillomas in these other occupations compared to the chrome platers; however this conclusion is reached without use of a statistical analysis.

In addition, Hanslian et al. state that the papillomas showed no signs of atypical growth or malignant degeneration. This study does not provide strong evidence that occupational exposure to Cr(VI) is associated with oral mucosa squamous cell carcinomas, the tumors observed in the high-dose NTP study rats. It is also important to note that from 1983 through 1987, Czechoslovakian males had the second highest rate of oral cavity cancer and pharyngeal cancer incidence in the world (Blot et al. 1996).

Oral papillomas, a subset of oral cancers, are most commonly caused by the human papilloma virus (HPV), a sexually transmitted disease (Syrjanen 2003 & 2005; Cheah et al. 1998). HPV is common in human populations, and some forms of the virus have a greater chance of causing dysplasia, which may lead to the development of cancer. Further, there is an interaction between smoking/chewing tobacco and the HPV virus, such that individuals with tobacco exposure who have the virus are at higher risk of developing cancer than persons who have either risk factor alone.

Additionally, the Hanslian et al. (1967) reported higher levels of chromium in the papillomas than in the tissues of former workers and unexposed individuals. However, this observation is expected, given the ongoing high level of airborne exposure incurred by these workers. Finally, with regard to this 1967 paper specifically, there are inconsistencies in the numbers of papillomas presented in various locations throughout the paper. While the text states that 14 individuals had papillomas, Table 2 in the paper reports 11 individuals with papillomas, and Table 6 of the paper provides information for 10 individuals with papillomas.

Studies of Oral Cavity Effects in Other Occupational Exposure Studies

With regard to the relevance of oral cavity observations in Cr(VI)-exposed workers, NTP should consider two additional studies, Miller 1950 and Public Health Service (PHS) 1953. These studies both examined the oral cavity of workers from the historical chromate production industry in the early 1950s, an industry with extraordinarily high airborne exposures and obvious oral exposures (Figure 3, Reproduced from PHS 1953). Approximately 40% of these

workers had discoloration of the anterior teeth, tongue, and dorsum from airborne Cr(VI) exposure (PHS 1953). Exposures in these plants were very high compared to current occupational exposures and generally consistent with those reported for the Hanslian et al. (1967) study. However, neither Miller (1950) or PHS (1953) found an increase in oral tumors (including papillomas) upon clinical inspection, although irritation effects in the nasal mucosa, tongue, and throat were common.

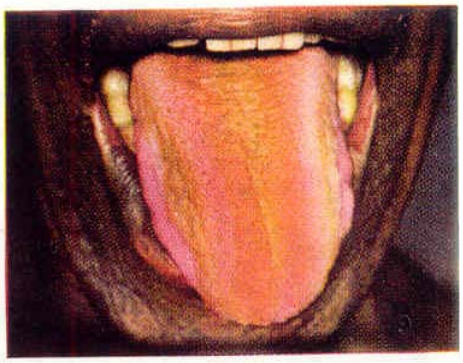

Figure 11.—Discoloration of tongue of a chromate worker.

Figure 3. Photograph of yellow-discolored tongue in a chromate production worker (from PHS 1953)

Considerations Regarding Zhang and Li (1987) Study of Chinese Villagers

NTP cites a 1987 study of Chinese villagers exposed to Cr(VI) in drinking water to support an association between Cr(VI) ingestion and stomach cancer (Zhang and Li 1987). However, this study has several limitations that should be considered before it is used as evidence of such an association.

- First, it is more likely that disease preceded exposure, rather than exposure preceding disease. The plant began smelting chromium in 1961. In 1965, drinking-water wells were contaminated in the closest village, and in the distant villages by 1974. However, cancer mortality data were collected only for the period of 1965–1978; thus, the latency period between exposure and death for this study ranges from only 4 to 13 years. The latency period for chemical carcinogenesis and stomach cancer ranges from 15 to 50 years (Plummer et al. 2004; Steenland and Palu 1999). Hence, there is a temporal mismatch between potential exposure and stomach cancer mortality. It is therefore biologically implausible that Cr(VI) in drinking water was a causative factor in the reported stomach cancer mortality.

- Stomach cancer is, and was at the time of the study, the most common form of cancer in China. Only one of the villages studied by Zhang and Li (1987)

had a higher rate of stomach cancer for the years of 1970–1978 compared to the average rate for China.

- Possible confounding from differences in gender and effects of smoking, alcohol consumption, dietary factors, socioeconomic status (SES), and occupation are all relevant to stomach cancer risk but were not considered by Zhang and Li (1987).

- The ecologic study design of Zhang and Li (1987) did not assess whether the villagers drank the contaminated water. This is particularly important, because a sizeable proportion of wells in the villages had minimal contamination, and a significant proportion had no contamination detected at all. Thus, it is questionable whether the villagers chronically consumed the contaminated water.

For these and other reasons not described in detail in these comments, the Zhang and Li (1987) study does not provide credible evidence that Cr(VI) is associated with stomach cancer.

While there are no definitive epidemiologic studies on humans exposed to Cr(VI) in drinking water, NTP may want to consider Fryzek et al. (2001) as an alternative study to Zhang and Li (1987). Fryzek et al. (2001) found no increase in cancer risk among a population with exposure to Cr(VI) in drinking water. While this study also has an ecological design and limited exposure information, the epidemiologic methods are current and scientifically sound.

Reviews of Epidemiologic Literature Cited by NTP

NTP cites reviews by Cohen et al. (1993), Costa (1997), and Costa and Klein (2006) in support of an association between Cr(VI) and cancers in humans outside the respiratory tract. NTP should be aware of the many inaccuracies in the reporting of the original studies in these reviews. For example, in Costa (1997), the data for brain cancer in the studies by Moulin et al. (1990), Dalager et al. (1980), Becker et al. (1991), and Deschamps et al. (1995) are presented as bone cancer in Costa (1997). In addition, the observed-to-expected ratio for stomach cancer in Deschamps et al. (1995) is reported incorrectly.

Cohen et al. (1993) cites Langard et al. (1980) as an epidemiologic study of kidney cancers, although kidney cancer was not investigated in the paper. Further, Cohen et al. cites Paustenbach et al. (1991) and Sheehan et al. (1991) as epidemiologic studies of chromium-induced lung cancers, neither of which are epidemiologic studies.

These reviews are misleading, because both Costa (1997) and Cohen et al. (1993) report no statistical analyses (confidence intervals), and many of the SMRs cited are based on very small sample sizes and are not statistically significant. For example, in Costa (1997), none of the SMRs presented for prostate cancer, lymphoma, leukemia, or bone cancers are statistically significantly elevated. As discussed in more detail below, Cole and Radu (2005) conducted meta-analyses for several of these cancers and reported no statistically significant increase. Because the relevance of the NTP study results to Cr(VI)-exposed humans is of great societal

impact, NTP should take extraordinary caution in citing these reviews, due to the misrepresentation and misreporting of findings in the epidemiology literature.

As an alternative to these references, we suggest that NTP consider the recent findings of Cole and Radu (2005). These authors conducted exhaustive meta-analyses of 49 epidemiologic studies based on 84 papers published since 1950. The meta-analyses showed that SMRs for lung cancer were statistically elevated. The SMR for stomach cancer was elevated (SMR = 113; CI 103–124) for all studies combined. However, when studies that controlled for social economic status (SES) were analyzed together, the SMR decreased to 82 (CI 69–96). As lower SES is a known risk factor for stomach cancer, the slightly elevated stomach cancer SMR among all studies was attributed entirely to the lack of SES control in some studies. In Cole and Radu's extensive review of the literature using methods to consider statistical significance, no other cancers evaluated were significantly elevated, including prostate, kidney, central nervous system, leukemia, Hodgkin's disease, or other lymphatohematopoietic cancer. NTP may also want to consider Barceloux (1999), which is an additional review article identifying some of the errors in the Costa (1997) review.

Inter-Species Variability

Inter-species variability in tumor outcome in the NTP study is notable, and differences in kinetics between rats and mice are important in understanding the mode-of-action for small intestine tumors in mice, and extrapolation of results to humans. The tissue data reported in Appendix J of the NTP study show that mice dose-responsively had higher amounts of Cr in the liver, as well as in plasma and erythrocytes compared to rats (Figure 4). Further, rats had a higher mass of Cr in feces than mice. This occurred on both a total mass basis (as reported in Appendix J of the NTP study) and when the mass of Cr in feces is adjusted for body weight (an approximate measure of food consumption) (Figure 5). To compare the feces chromium content of male rats and female mice, each mass of chromium in feces (μg) was divided by the estimated mean body weight (g) on Days 7, 14, 175, and 371 in Figure 5. This was conducted to adjust for the difference in fecal mass by species because chromium concentration in feces was not provided.

These kinetic differences are important in understanding inter-species variability, because Cr(III) is not readily absorbed and thus excreted via the feces, whereas Cr(VI) is more readily absorbed into tissues (Gargas et al. 1994). Also, of the tissue data collected in the NTP study (Appendix J), tissue levels in the liver are most representative (on a relative basis) of chromium levels in small intestine tissues, due to the uptake of chromium through portal circulation. Thus, it is reasonable to conclude that more Cr(VI) was reduced to Cr(III) in the stomach of the rat and passed unabsorbed as Cr(III) in the feces, than in the mouse. Similarly, less Cr(VI) was reduced to Cr(III) in the stomach of the mouse and passed to the small intestine, where it caused tissue damage and was taken-up into the liver. Thus, the tissue dose in the small intestine of the mouse was likely greater than that in the liver. These kinetic differences between species are important in understanding the mode-of-action.

Histiocytic infiltrates in the liver, mesenteric and pancreatic lymph nodes, and small intestine are also consistent with the known toxicology of Cr(VI), while also indicating important differences between rats and mice. Histiocytic infiltrates represent an immune response that occurs at overwhelmingly high doses of Cr(VI) and chromium accumulation in these tissues.

Finally, we request the review board's comments on the possible biological basis for the observed inter-species variability observed in the NTP study. We have noted that the basal gastric excretion rates range from 0.96 μEq gastric acid/4 hours in wildtype AQP4 mice (Wang et al. 2000) to 168 μEq/4hr in wildtype C57BL16 mice (Friis-Hansen et al. 1988). Rats have a greater basal gastric acid production rate (1,200 μEq/4h) (Runfola et al. 2003), and humans have a far greater gastric acid production rate (8,000-20,0000 μEq/4h) (de Zwart et al. 1999). While basal gastric acid production is expected to be greater in rats than mice based on species size, the tissues of the stomach in each species was exposed to the same concentrations of Cr(VI) in the NTP study. This physiological difference may explain the observed differences in Cr(VI) reduction kinetics and toxicology in rats and mice in this study.

Chronic Irritation as a Mode-of-Action for Small Intestinal Tumors in Mice

In the NTP study, intestinal tumors (adenomas and carcinomas) were observed in both male and female mice. Epithelial hyperplasia and histiocytic infiltration were reported as the predominant nonneoplastic lesions in the intestine. These observations were dose-dependent with the increase in epithelial hyperplasia occurring at lower doses than the increase in tumor formation, suggesting a causal relationship. Cr(VI) is a known skin and mucous membrane irritant (Barceloux 1999). In the following paragraphs, we present information to support that chronic irritation of the intestinal mucosa caused by high doses of Cr(VI), or other factors including accumulation of Cr(III) in tissues and physiological alterations associated with dehydration, may have caused the epithelial hyperplasia and the subsequent formation of intestinal tumors.

Chronic irritation of tissues causes hyperplasia, metaplasia, and dysplasia which are nonneoplastic responses of tissues that can progress to benign and malignant tumors (Cotran et al. 1994, Quah et al. 2005, Wilkinson and Killeen 1996). For example, chronic dietary treatment of rodents with the fungicide chlorothalonil causes an increased incidence of papillomas and carcinomas of the forestomach squamous epithelium, as well as adenomas and carcinomas of the kidney, in both rats and mice (Wilkinson and Killeen 1996). There is strong evidence suggesting that these tumors occur through a non-genotoxic mechanism – specifically, chronic irritation and cytotoxicity followed by compensatory cell proliferation and hyperplasia.

While there is limited information to evaluate small intestinal tumors, in the rodent forestomach continued irritation of squamous epithelium causes cytotoxicity, resulting in inflammation, ulceration, and regenerative hyperplasia (Wilkinson and Killeen 1996). Many studies have shown that chronic irritation caused compensatory epithelial hyperplasia, inflammation, and carcinomas of the forestomach. Such cell proliferation and hyperplasia are typically threshold-based effects and, in the initial stages, are usually reversible with cessation of exposure (Kagawa et al. 1993).

Importantly, chronic irritation of epithelial tissue is generally not consistent with human exposure conditions, which are likely below a threshold for irritation. Further, cytotoxicity associated with high dose exposure likely accelerates the rate of tumorigenesis and may be an important consideration in quantifying the dose-response. For example, it is possible that a threshold for human cancers may exist at doses below those that cause non-neoplastic lesions in the forestomach (Proctor et al. 2007).

It is becoming increasingly recognized that prolonged chemical-induced cell proliferation can cause tumor formation in animals from high dose exposures. Examples outside the forestomach include chloroform in the liver, sodium saccharin on the bladder, and ethylene thiourea on the thyroid (Wilkinson and Killeen 1996). Further, several studies suggest that the natural history of esophageal cancer may start with esophagitis, evidenced by the progression of atrophy and displasia of the epithelium, and finally neoplasia (reviewed in Ribeiro et al. 1996). Some of the causes of esophagitis reviewed include consumption of extremely hot beverages, betel nuts, and opium smoking.

A similar phenomenon is also observed in humans, where chronic chemical irritation, mechanical irritation, and inflammation may ultimately predispose an organ to cancer formation. For example, inflammatory bowel disease in the terminal ileum and colon (Crohn's disease, ulcerative colitis) is associated with colorectal carcinoma, and reflux esophagitis—due to chronic reflux of gastric acid and bile into the esophagus—has been associated with esophageal carcinoma (Coussens and Werb 2002; Ryan 1996; Thun et al. 2004). Similarly, chronic cholecystitis and gallstones have developed into cancer of the gall bladder; nodular lymphoid hyperplasia has developed into small intestinal lymphoma; and celiac sprue is a premalignant condition for small intestinal lymphoma and adenocarcinoma (Ryan 1996; Thun et al. 2004). In all of the chronic diseases mentioned above, physical or chemical irritation and/or chronic inflammation play a key role in predisposing the tissue to the formation of benign and malignant tumors.

In the description of the histopathology of epithelial hyperplasia in the current NTP study, NTP compares the observed nonneoplastic finding to intestinal adenomas, stating, "this [focal epithelial hyperplasia] was considered a preneoplastic lesion related to exposure to sodium dichromate dehydrate because of its morphologic similarities to adenoma" (page 74 of NTP study report). It is probable that high doses of Cr(VI), a known mucous membrane irritant, caused epithelial hyperplasia in the intestine and progressed to the formation of benign and malignant intestinal tumors. Thus, the mode-of-action for intestinal tumors in mice may have involved a non-genotoxic irritation mechanism.

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Tables 1 and 2

Table 1. Results from epidemiologic studies that evaluated occupational exposure to Cr(VI) and risk of oral cancers

First Author, Year	# Cases or Deaths	SMR	95% CI
Becker 1999 (lip, oral cavity, pharynx)	1	0.32	(0.08, 1.76)
Birk 2006 (oral cavity, pharynx)	1	0.49	(0.01,2.74)
Blair 1980 (buccal cavity, pharynx)	11	1.45[b]	(0.72, 2.59)
Boice 1999 (buccal cavity, pharynx)	1	0.14	(0, 0.77)
Dalager 1980 (buccal cavity, pharynx)	3	2.5[b]	(0.52, 7.31)
Davies 1991 (mouth, pharynx)	6	2.17	(0.79,4.71)
Deschamps 1995 (pharynx)	1	0.52	(0.01, 2.87)
Gibb 2000 (buccal cavity, peritoneum)	8	1.04	(0.21, 3.05)
Guberan 1989 (buccal cavity, pharynx)	7	1.84	(0.86, 3.46)
Montanaro 1997 (oral cavity, pharynx)	4	1.24	(0.34, 3.18)
Moulin 1990 (buccal cavity, pharynx, larynx)	7	0.91	(0.37, 1.88)
Moulin 1993 (buccal cavity, pharynx)	6	0.84	(0.31, 1.84)
Rafnsson 1997 (lips)	2	0.87[a]	(0.10, 3.13)
Silverstein 1981 (buccal cavity, pharynx)	1	0.77[b]	(0.02, 4.32)
Simonato 1991 (buccal cavity, pharynx)	3	0.44	(0.09, 1.29)
Sorahan 1987 (buccal cavity and throat)	2	0.95	(0.11, 3.44)
Sorahan 1994 (lip)	0	0	(-)
Sorahan 1994 (tongue)	4	1.29	(0.35, 3.31)
Sorahan 1994 (salivary gland)	0	0	(-)
Sorahan 1994 (mouth)	3	1.02	(0.21, 2.99)
Sorahan 1994 (pharynx)	6	0.93	(0.34, 2.03)

[a] SIR

[b] PMR

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Table 2. Results from epidemiologic studies that evaluated occupational exposure to Cr(VI) and risk of intestinal cancer

First Author, Year	# Cases or Deaths	SMR	95% CI
Axelsson 1980 (small intestine, colon)	3	0.53	(0.11,1.56)
Birk 2006 (intestine, except rectum)	4	1.08	(0.29,2.76)
Iaia 2006 (small intestine)	1	2.04	(0.05, 11.4)
Montanaro 1997 (small intestine, colon)	10	1.66	(0.8, 3.05)
Moulin 1990 (intestine, except rectum)	0	0	(0, 3.1)
Moulin 1993 (intestine)	1	0.33	(0.01, 1.84)
Rafnsson 1997 (small intestine)	3	4.23	(0.85, 12.35)
Simonato 1991 (intestine, except rectum)	17	1.18	(0.69, 1.89)
Sorahan 1994 (small intestine)	1	0.55	(0.01, 3.04)

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Sorahan, T., A.M. Faux, et al. 1994. Mortality among a cohort of United Kingdom steel foundry workers with special reference to cancers of the stomach and lung, 1946–90. Occup. Environ. Med. 51(5):316–322.

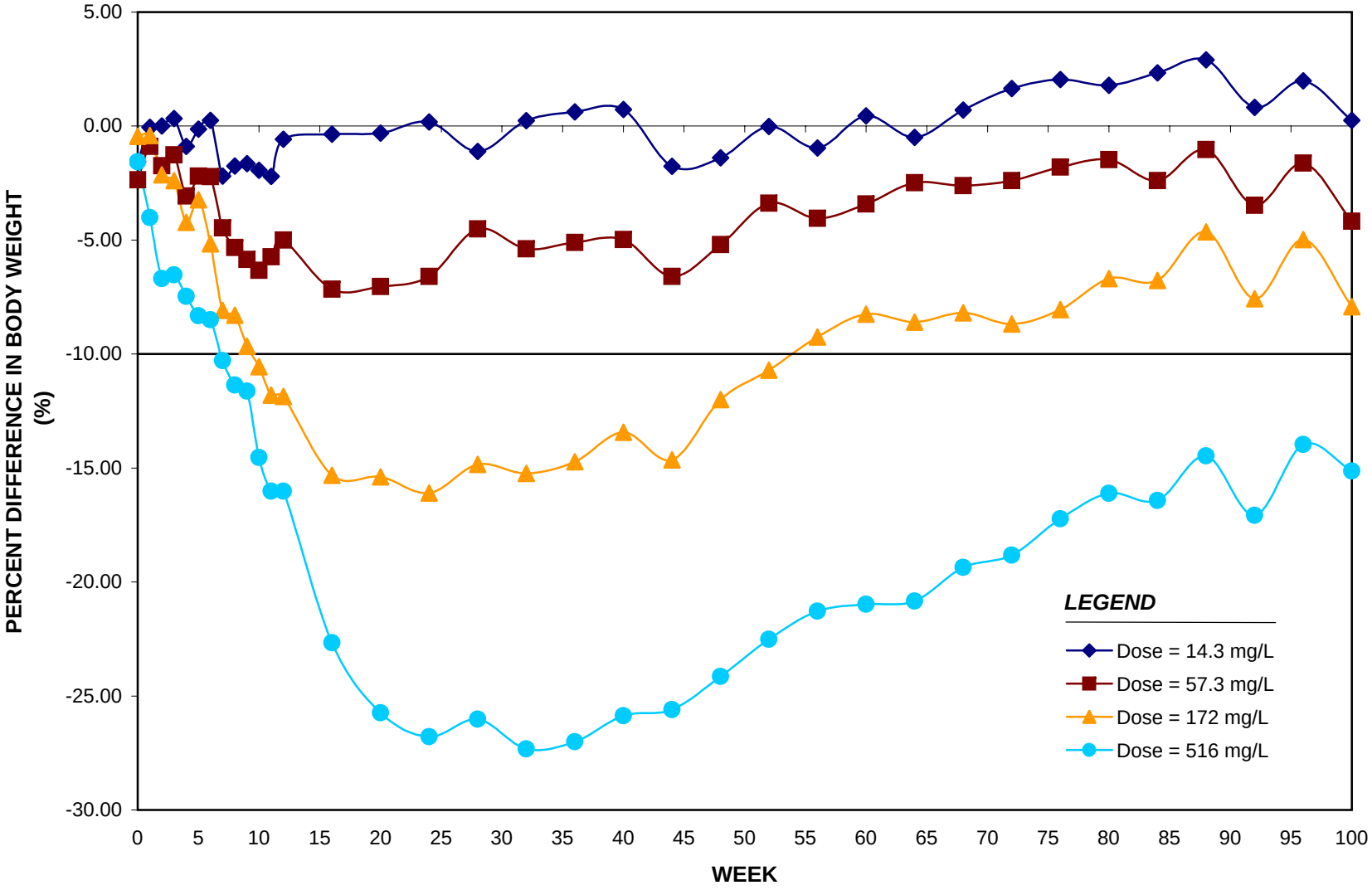


Figure 1a. Percent difference in body weight from control: Female mice

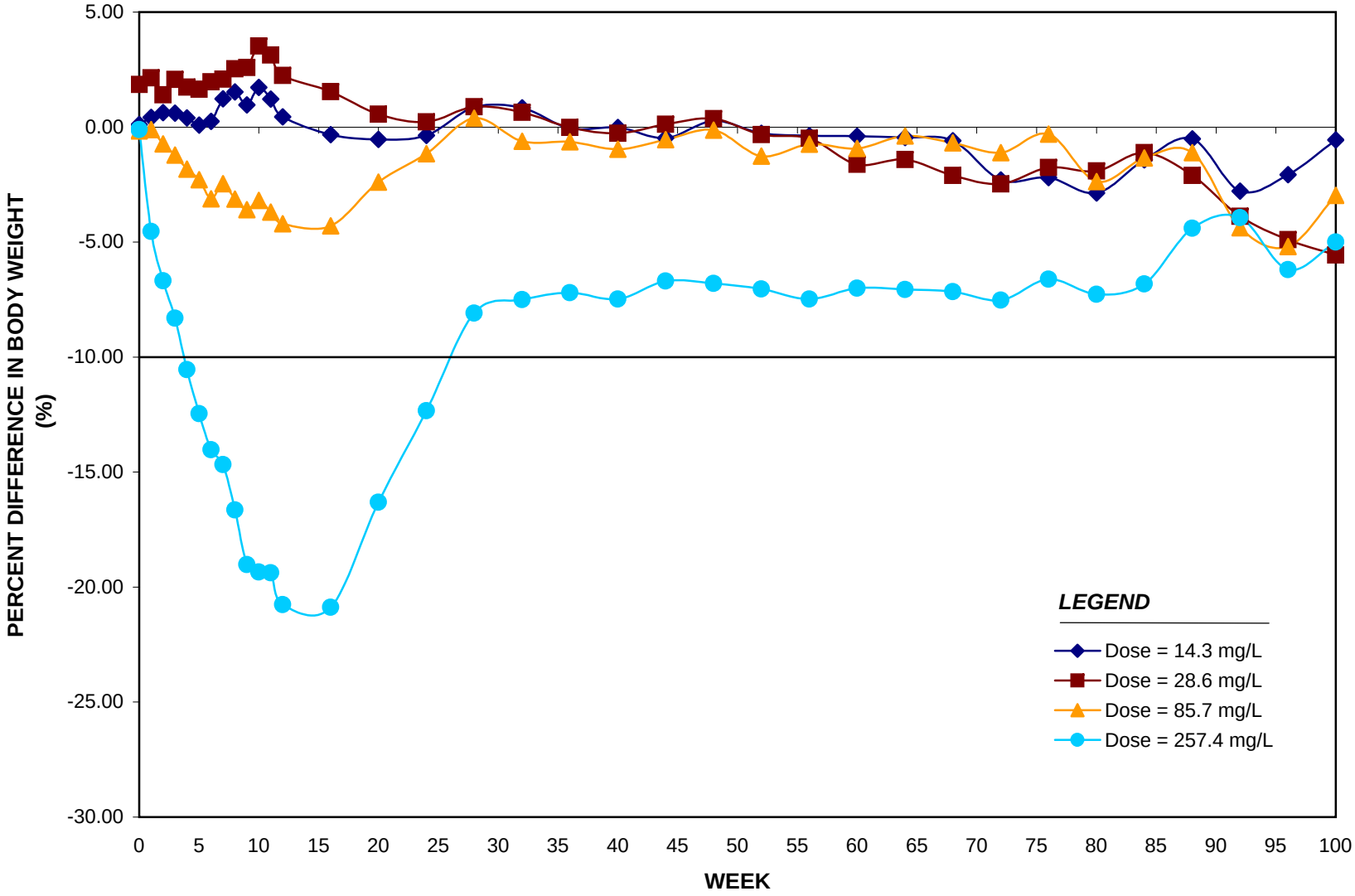
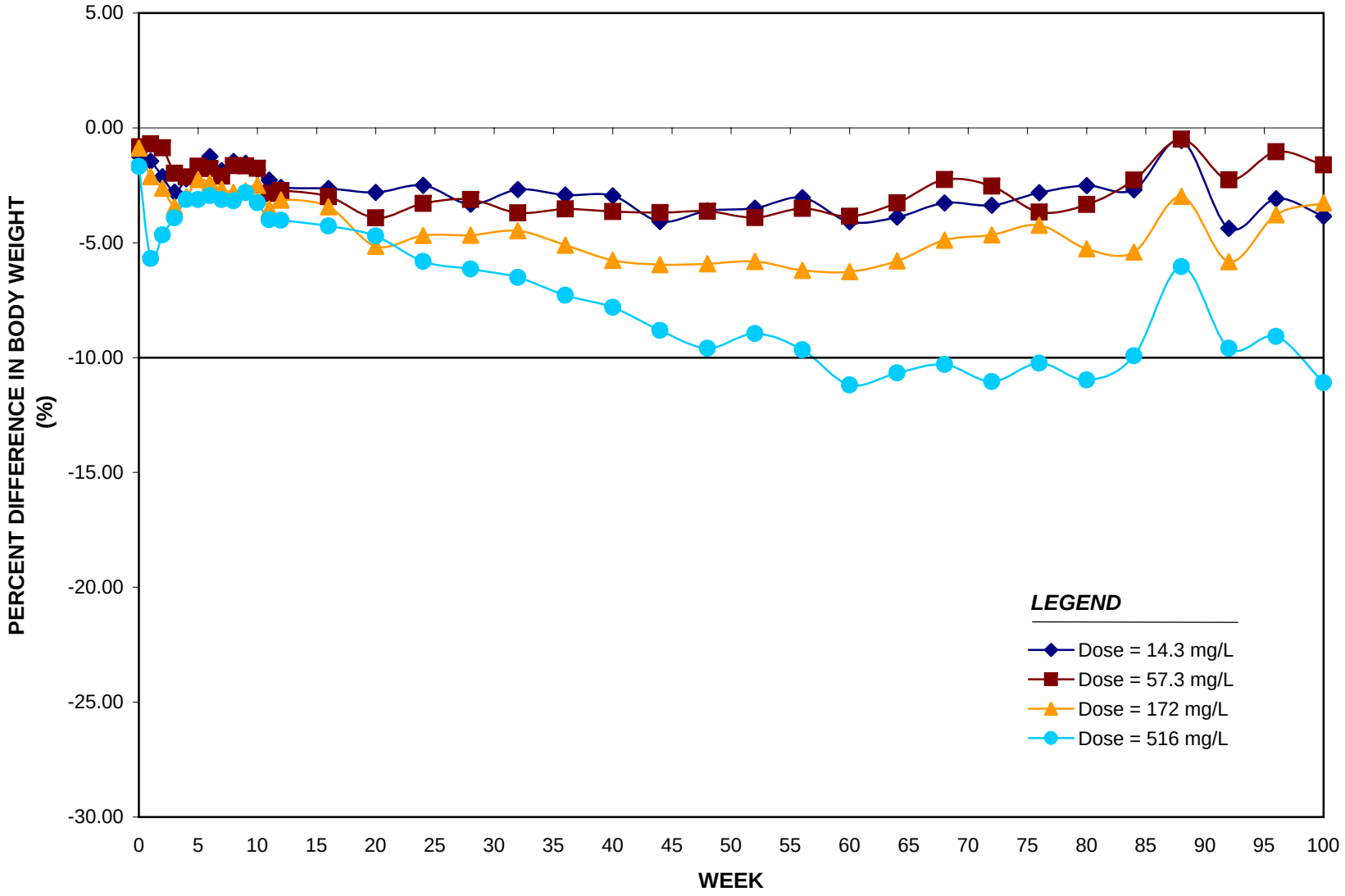


Figure 1b. Percent difference in body weight from control: Male mice



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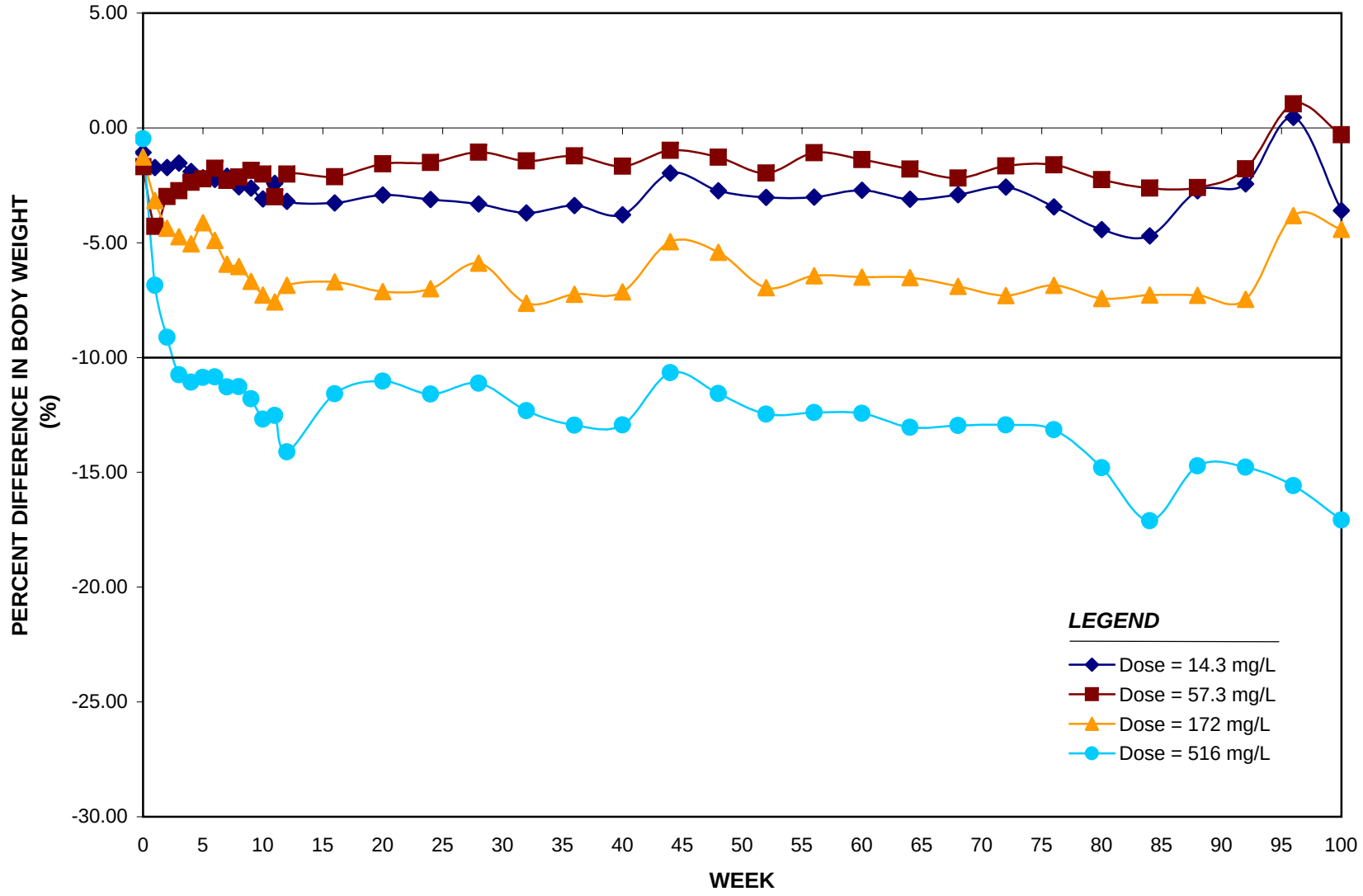

Figure 1d. Percent difference in body weight from control: Male rat

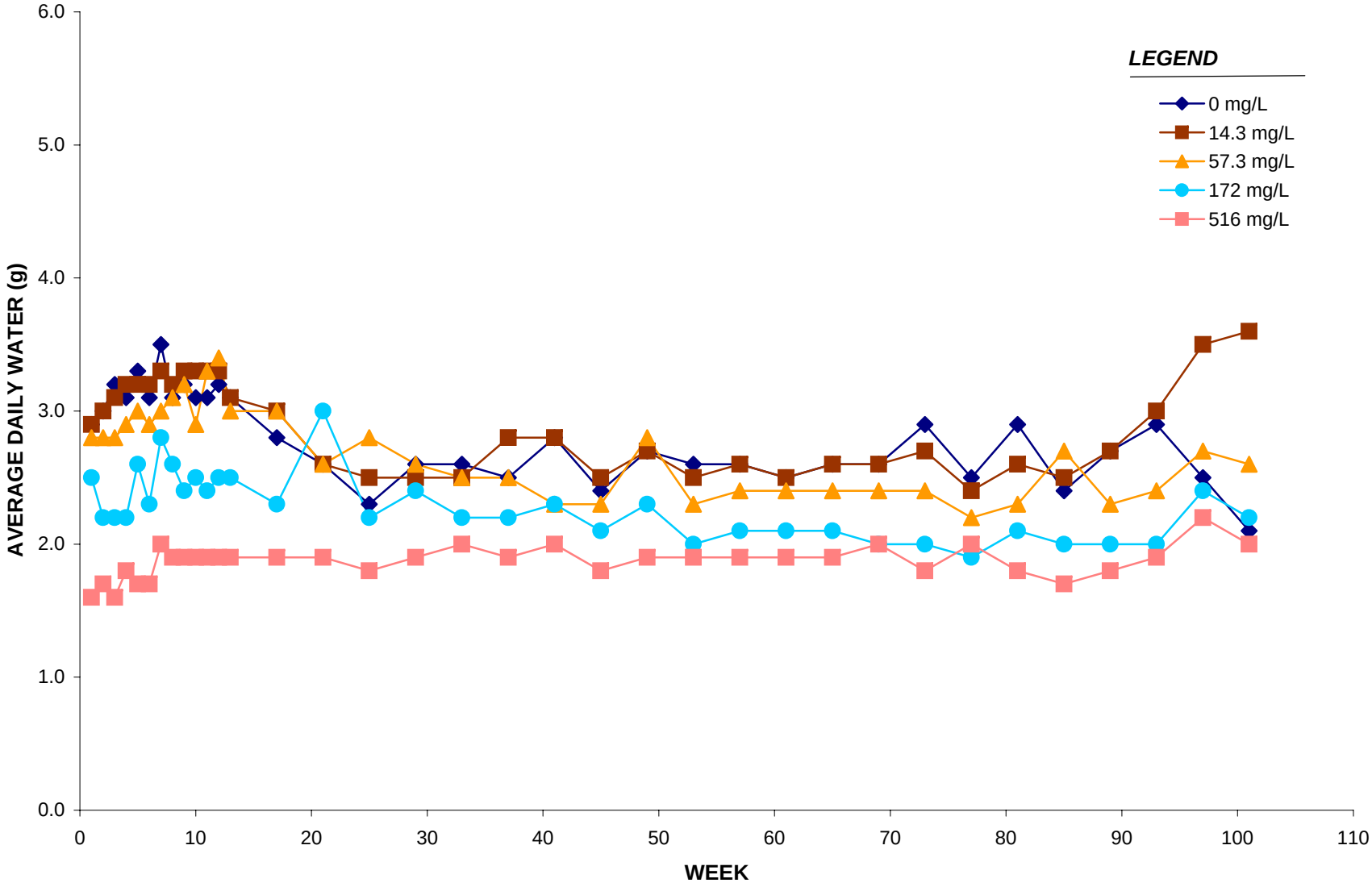

Figure 2a. Average daily water consumption: Female mice

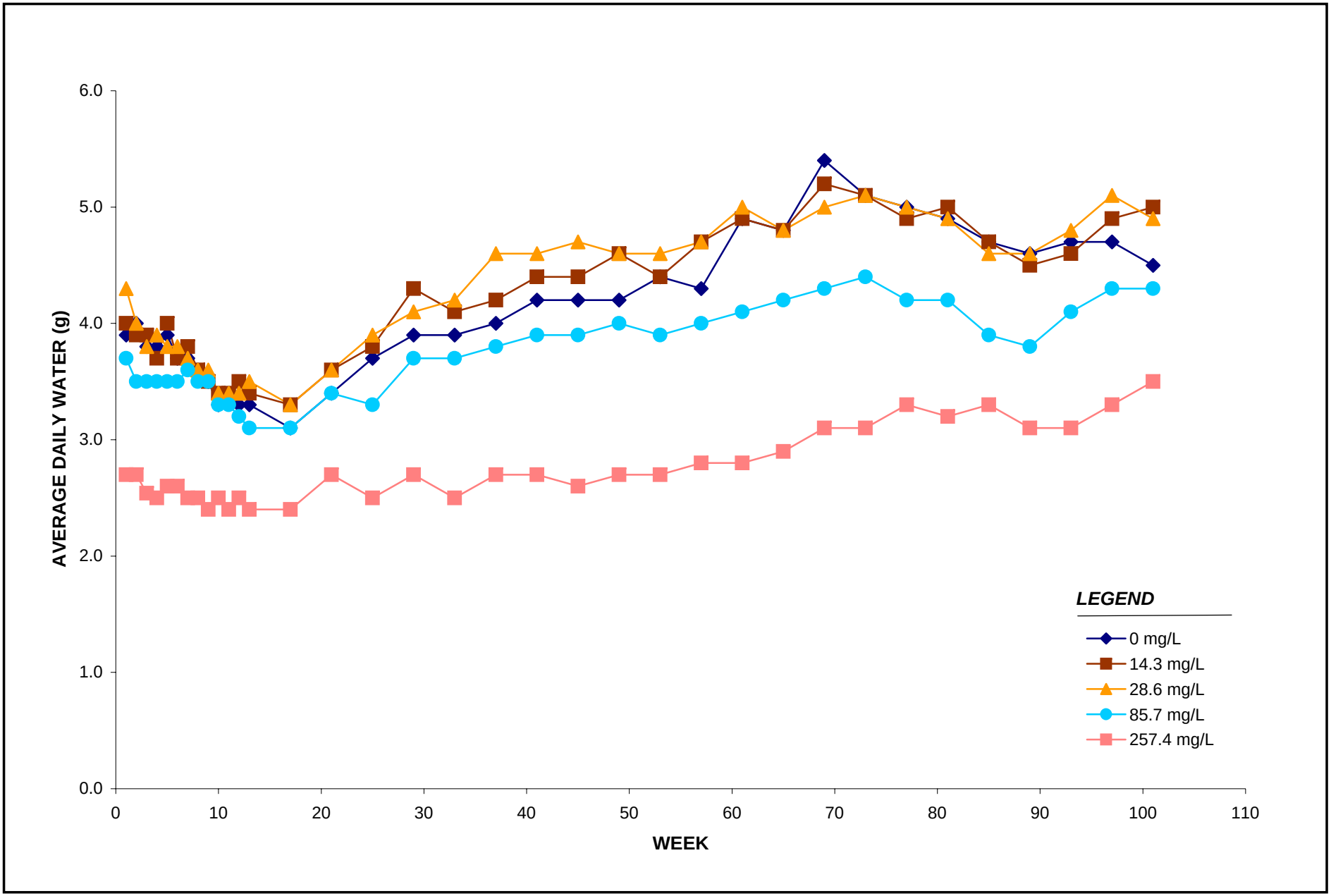

Figure 2b. Average daily water consumption: Male mice

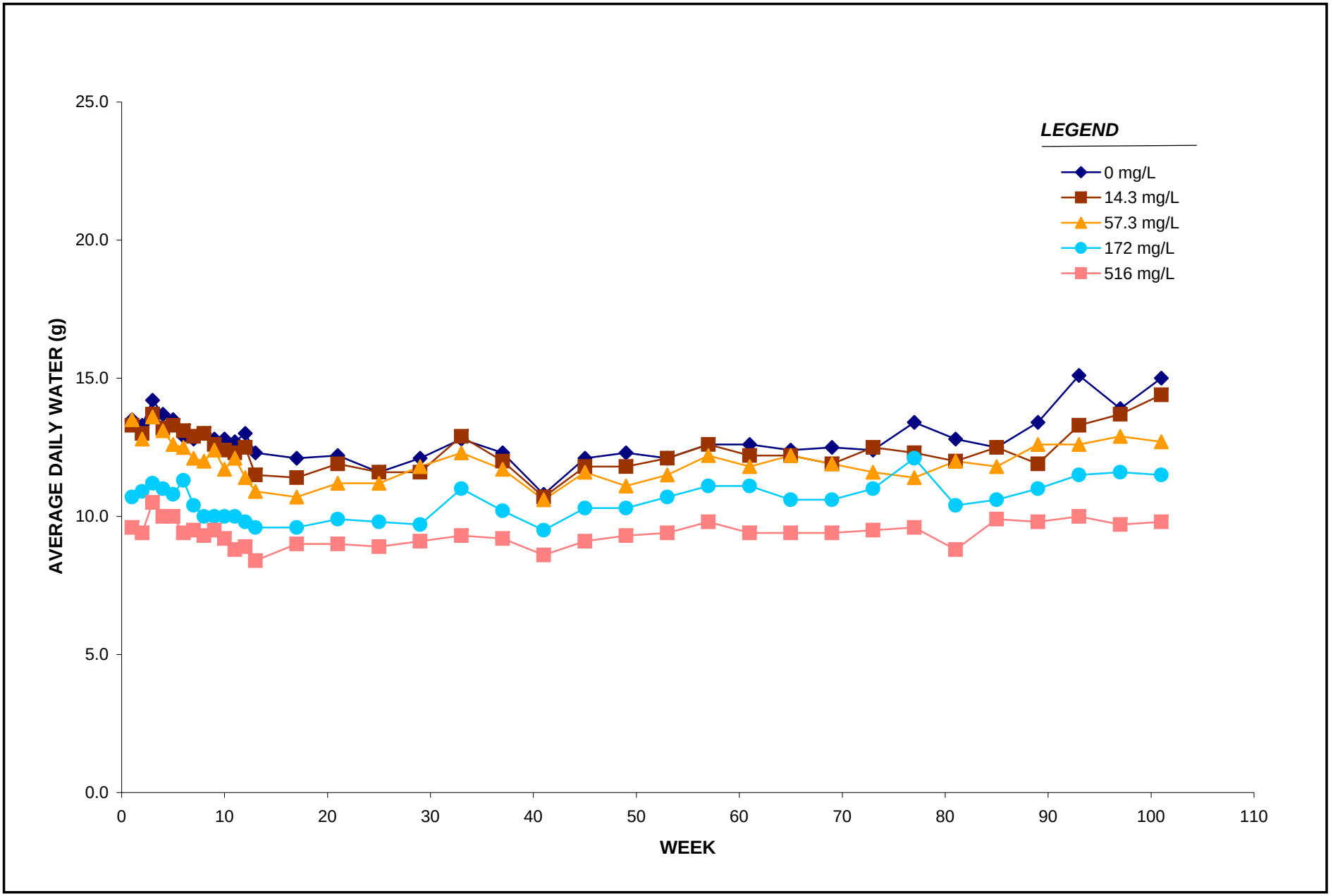

Figure 2c. Average daily water consumption: Female rats

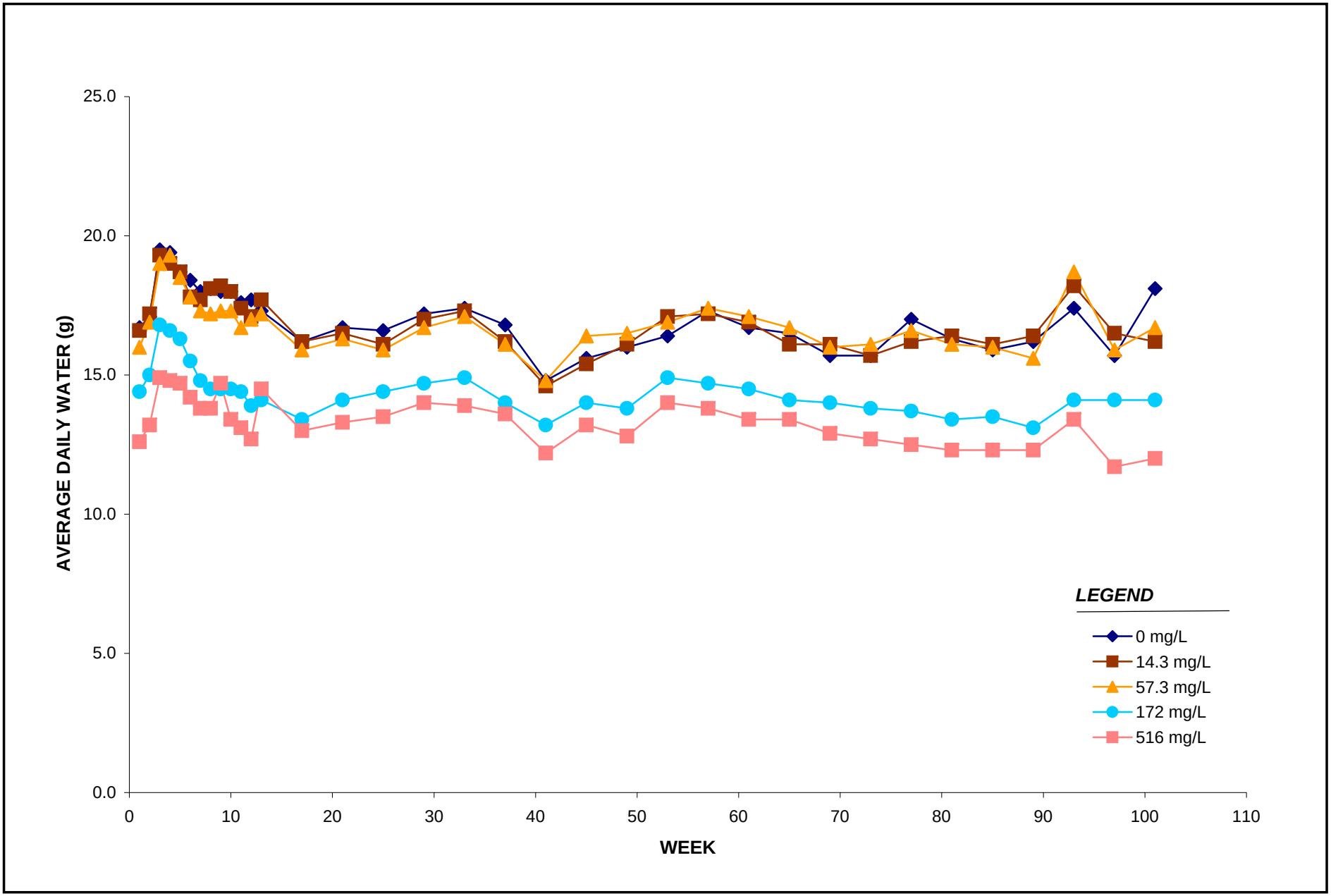

Figure 2d. Average daily water consumption: Male rats

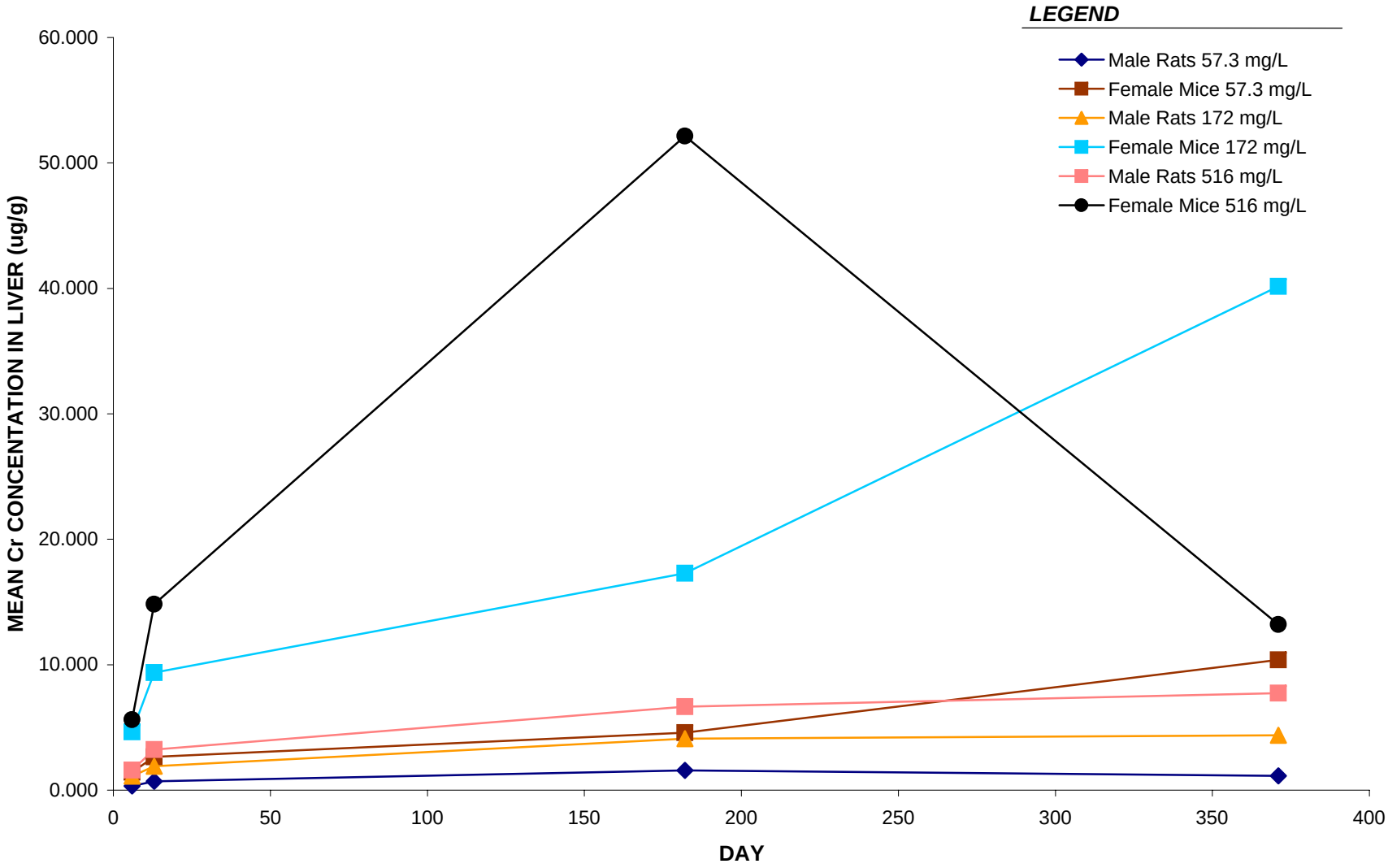

Figure 4. Mean chromium concentrations in liver of NTP female mice and male rats by dose group

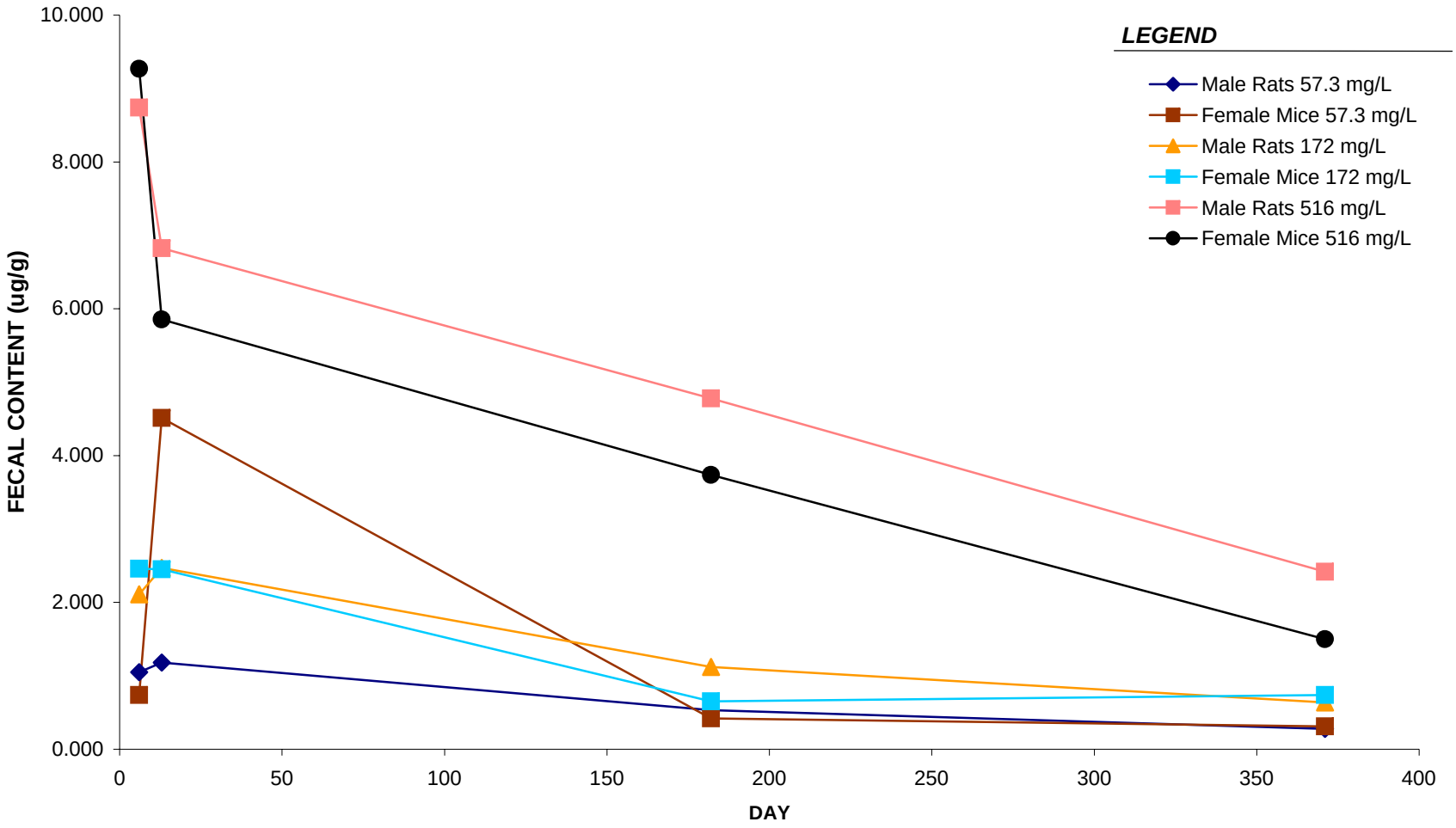

Figure 5. Estimated chromium concentrations in feces of female mice and male rats by dose group