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Andrea Chambers

AUTEUR DE LA THÈSE / AUTHOR OF THESIS

M.Sc. (Epidemiology)

GRADE / DEGREE

Department of Epidemiology and Community Medicine

FACULTÉ, ÉCOLE, DÉPARTEMENT / FACULTY, SCHOOL, DEPARTMENT

The Application of Categorical Regression to Model the Exposure-Response Relationship of Copper Excess and Deficiency

TITRE DE LA THÈSE / TITLE OF THESIS

N. Birkett

DIRECTEUR (DIRECTRICE) DE LA THÈSE / THESIS SUPERVISOR

CO-DIRECTEUR (CO-DIRECTRICE) DE LA THÈSE / THESIS CO-SUPERVISOR

EXAMINATEURS (EXAMINATRICES) DE LA THÈSE / THESIS EXAMINERS

Y. Chen

B. Choi

Gary W. Slater

Le Doyen de la Faculté des études supérieures et postdoctorales / Dean of the Faculty of Graduate and Postdoctoral Studies

THE APPLICATION OF CATEGORICAL REGRESSION TO MODEL THE EXPOSURE-RESPONSE RELATIONSHIP OF COPPER EXCESS AND DEFICIENCY

ANDREA CHAMBERS

Thesis submitted to the Faculty of Graduate and Postdoctoral Studies in partial fulfillment of the requirements of the MSc degree in Epidemiology

Epidemiology and Community Medicine
Faculty of Medicine
University of Ottawa

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Abstract

There is a need to define an exposure-response curve for both copper excess and deficiency to assist in defining the acceptable range of oral intake. A copper exposure-response database has been developed where response data has been assigned to ordinal severity scores. A generalized linear model was used to estimate the probability of response associated with dose, duration and severity. The exposure-response model is defined to account for differences in animal species, route of exposure and age. The exposure-response curves for copper excess and copper deficiency have defined an optimal intake level of 2.0 mg Cu/day and an acceptable range of oral intake between 1.8 and 3.1 mg Cu/day. These results suggest that current recommendations for copper intake including the recommended dietary intake (0.9 mg/day) and the tolerable upper intake level (10 mg/day) may not protect the population from responses that might occur outside the limits of the homeostatic range.

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TABLE OF CONTENTS

1. LIST OF TABLES

1. LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviations or Symbols	Terms
AIC	Akaike information criteria
AROI	acceptable range of oral intake
APOE4	apolipoprotein E4
BMD	benchmark dose
BMI	body mass index
ATOX1	antioxidant 1
ATP	adenosine-5'-triphosphate
BW	body weight
C	concentration
CCO	cytochrome C oxidase
CI	confidence interval
C log-log	complementry log-log
CTR1	copper transport protein
Cu	copper
df	degrees of freedom
EAR	estimated average requirement
ER	extra risk
ERC	extra risk concentration
GI	gastro intestinal
H	cumulative probability function
HDL	high density lipoprotein
HU	humans
LDL	low-density lipoprotein
LOAEL	lowest observed adverse effect level
MU	mice
NOAEL	no observed adverse effect level
PAM	peptidylglycine α-amidating monooxygenase
q	extra risk

R^2	coefficient of determination
RDI	recommended dietary intake
RfD	reference dose
RR	relative risk
RT	rats
S/SEV	severity score
SD	standard deviation
SOD1	Cu, Zn superoxide dismutase
TI	tolerable upper intake level

PART 1: INTRODUCTION AND REVIEW

Copper is one of a number of metallic elements including, zinc, iron, chromium, selenium, cobalt, iodine, and molybdenum that have been identified as having vital physiological functions within the body (WHO, 2002). The World Health Organization (WHO) categorizes a metal as essential when "absence or deficiency of the element from the diet produces either functional or structural abnormalities and that the abnormalities are related to, or a consequence of, specific biochemical changes that can be reversed by the presence of the essential metal (WHO, 1996)." Like all other elements, too much copper can also lead to undesirable toxic effects. While the body has a complex regulatory system to maintain internal concentrations of copper within an appropriate range, it is possible to overwhelm these adaptive mechanisms resulting in toxicity due to either deficiency or excess (Aggett and Fairweather-Tait, 1998). In order to provide a background on copper risk assessment issues, the following topics will be discussed: copper's role in the body; copper solubility; adverse effects associated with copper deficiency and excess; the evidence associating copper with chronic disease; populations at increased risk; copper homeostasis; typical exposures and nutritional reference values; and challenges in copper exposure-response assessment.

1.1 COPPER'S ROLE IN THE BODY

Copper is a micronutrient that has an essential role in the functioning of several enzymes in the body. Copper's primary role is catalytic as several copper metalloenzymes act as oxidases which catalyze an oxidation or reduction reaction involving molecular oxygen (Food and Nutrition Board, 2002). Specifically, copper enzymatic reactions are involved in: normal utilization of iron; control of neurotransmitters and neuropeptides; the maintenance of bone; the maintenance of connective tissue in lungs, bone and elastin in the

cardiovascular system; oxidative metabolism; brain functioning; phospholipid synthesis; pigmentation; and detoxification of superoxide radicals (Stern et al., 2007). Table 1.1 lists enzymes and biological processes where copper plays a central role (Harvey and McArdle, 2008). Disruption of these activities is largely responsible for the clinical features of copper deficiency. Copper can also be toxic at high concentrations, potentially producing oxidative damage to biological systems including the peroxidation of lipids or other macromolecules (Bremner, 1998).

Table 1.1: Enzymes and Biological Process Associated with Copper

Function	*Enzyme Protein*
Iron mobilization	Caeruloplasmin (ferroxidase I), hephaestin
Antioxidant defense	Cu, Zn-superoxide dismutase (SOD1), caeruloplasmin, metallothionein
Cu transport	Caeruloplasmin, albumin, transcuprein, ATP7A, ATP7B, copper transport protein
Formation of connective tissue	Lysyl oxidase, cartilage matrix glycoprotein
Electron transport	Cytochrome C oxidase (CCO)
Blood clotting	Blood clotting factors V and VIII
Deamination of primary amines	Amine oxidases
Alpha-amidation of neuropeptides	Peptidylglycine monooxygenase
Pigment production e.g. melanin	Tyrosinase
Catecholamine metabolism	Dopamine B-monooxygenase
Oxidation of phenylalanine to tyrosine	Phenylalanine hydroxylase
Metal detoxification	Glutathione
Copper chaperones	Antioxidant 1 (ATOX1): delivery of Cu to ATP7A and ATP7B Copper chaperone: delivery of Cu to SOD1 Cox 17: delivery of copper to CCO in mitochondria

1.2 COPPER SOLUBILITY

Human exposure to copper comes primarily from food and water. In drinking water, copper can be found as either ionizable copper or as copper combined with organic and

inorganic ligands (Pizarro et al., 2001). Some copper complexes have low solubility in water and will therefore be less bioavailable (Pizarro et al., 2001). Copper salts that have high solubility include copper sulfate, copper chloride, copper gluconate and copper acetate. These salts are generally more toxic than less soluble forms of copper including copper hydroxide, copper oxide and copper carbonate (Stern et al., 2007).

1.3 ADVERSE EFFECTS FROM COPPER DEFICIENCY & EXCESS

1.3A Copper Deficiency

While the U.S. Institute of Medicine's report on dietary reference intakes (Food and Nutrition Board, 2001) indicated that average intake levels of copper in the United States population are lower than recommended levels, there is a lack of public recognition and information on nutritional deficiencies from copper and their impact on health. While marginal copper deficiencies might be widespread, clinically evident copper deficiency is rare in humans. Evidence of copper deficiency in humans has primarily been derived from case reports, case series and depletion-repletion clinical studies. The most common responses to copper deficiency that have been investigated include: negative copper balance; cardiovascular disturbance; alteration of phospholipids; and changes in levels of glucose, insulin, immune parameters and enzyme levels. Moderate or severe copper deficiency is not common in the general population, except for low-birth weight or malnourished infants and adults receiving total parental nutrition without added copper (Stern et al., 2007; Cordano, 1978). Under these rare conditions of severe copper deficiency symptoms include normocytic and hypochronic anemia, leukopenia, neutropenia, and bone abnormalities (Fujita et al., 1989; Shaw, 1992). The maintenance of adequate levels of copper is essential during early development for normal growth, bone strength, production of red and white blood cells, iron transportation, and brain development. For infants and children, copper

deficiency may result in anaemia, leukopenia or bone alterations as well as increased incidence of infection and impaired weight gain (Cordano, 1978; Danks, 1988). One primary characteristic of copper deficiency is anemia, where deficient levels of copper impairs the function of ceruloplasmin (ferroxidase I) activity causing defective iron mobilization (Stern et al., 2007).

Currently, there is a lack of diagnostic criteria to assess marginal copper status; this is problematic as there is some evidence that prolonged marginal copper deficiency may increase one's susceptibility to infection, impair neurological function and elevate the risk of developing a range of diseases including heart disease and osteoporosis (IPCS, 1998; Klevay, 1980; Strain, 1994). The challenge is identifying an adequate biomarker to detect these marginal deficiencies. One of the limitations with traditional copper indices is that most of these measures (e.g., ceruloplasmin and plasma copper) are controlled by strong homeostatic mechanisms and can be influenced by factors unrelated to copper stores. Plasma copper levels do change throughout the day peaking in the morning (Solomons, 1979). Pregnancy and exogenous gonadal hormones stimulate ceruloplasmin production and raise copper levels (Halsted 1968; Hambidge et al., 1974; Horwitt 1975), whereas corticosteroids and the adrenocorticotropic hormone reduce copper levels (Yunice et al., 1981).

Indicators that have been used to detect states of copper deficiency include: serum copper and ceruloplasmin concentrations; erythrocyte superoxide dismutase activity; platelet copper concentration; cytochrome c oxidase activity; urinary copper; lysyl oxidase activity; and peptidylglycine α-amidating monooxygenase (PAM) activity (Food and Nutrition Board, 2001). Serum copper concentration is a reliable indicator of copper deficiency (Food and Nutrition Board, 2002; Danks, 1988); however, it does not reflect dietary intake unless intake is below a certain level. Another reliable indicator of copper deficiency is ceruloplasmin

concentration. Ceruloplasmin, the major copper-carrying protein in serum, decreases during copper deficiency and increases quickly following repletion (Food and Nutrition Board, 2002; Danks, 1988); however, like serum copper, it does not respond to marginal copper deficiency.

It has been suggested that erythrocyte superoxide dismutase (SOD) activity may be a more sensitive indicator of copper status (Milne, 1998; Uauy et al., 1985); however, it is not as specific as serum copper or ceruloplasmin concentrations (Food and Nutrition Board, 2001). Platelet copper concentration and platelet cytochrome c oxidase activity may respond more quickly to low dietary copper intake than serum copper, caeruloplasmin and SOD activity according to depletion-repletion studies on women (Milne and Nielsen , 1996).

Urinary copper excretion does not respond to increases in dietary copper (Food and Nutrition Board, 2001); however, in controlled studies, a decline in urinary copper excretion is observed when diets are low enough in copper (Turnlund et al., 1997). Lysyl oxidase has been considered as a potentially useful indicator of copper status (Werman et al., 1997) as it has been found to decline with low dietary copper intake and increase following repletion. There is recent and promising development in the identification of the copper chaperone for SOD1 (CCS) as a potential sensitive biomarker, although its reliability has not yet been established (Harvey and McArdle, 2008). Nonetheless, it is the most promising biomarker that responds to both copper deficiency and excess at this time (Harvey and McArdle, 2008).

1.3B Copper Excess

While copper deficiency might be more widespread, the U.S. National Research Council (2000) concluded in its report, "Copper in Drinking Water," that potentially susceptible subpopulations may be at risk of copper toxicity. Toxicity is largely due to

copper's ability to accept and donate electrons. As copper's catalytic activity is why it is so essential for a large number of enzymes, it will only be toxic when these activities are unsequestered and unmediated. Copper in excess can affect the bone, central nervous system and the immune system; however, the liver is the primary target organ of copper induced toxicity (International Programme on Chemical Safety, 1998).

At chronically elevated intakes, maladaptive reversible and irreversible responses may manifest depending on the magnitude and duration of the exposure. Repeated exposures can lead to tachycardia and respiratory disturbances, as well as histopathological changes in the liver and kidney (Stern et al., 2007).

Human studies on the long-term effects of chronic copper exposure are limited. A single human case study (O'Donohue et al., 1999) and numerous animal studies (IPCS, 1998) have demonstrated that chronic exposure to copper can lead to liver failure with different degrees of severity depending on the duration and degree of exposure to elevated levels of copper. In the case study, a young male who consumed 30-60 mg Cu/day for three years developed liver failure (O'Donohue et al., 1999).

Experimental evidence in animals suggests that excessive copper exposure can also lead to neurological disorders and reproductive effects. Whether these animal studies can be extrapolated to humans has not been established. Furthermore, the exposure levels used in the majority of experimental studies on animals are far beyond normal exposure levels in humans (IPCS, 1998). There is limited data available on the association between copper and reproduction and development in humans. Birth weight has been found to be negatively correlated with maternal copper levels (Ozdemir et al., 2007). In a small study looking at trace element status and birth outcomes, there was a significant positive correlation between the copper/zinc ratio and birth weight (Mbofung and Subbarau, 1990). There was also a

study of women in Massachusetts in which no associations were found between the risk of spontaneous abortions in women exposed to drinking water with greater than 1 mg Cu/L (Aschengrau et al., 1989). There is currently no reliable biomarker for copper excess resulting from dietary exposures. At this time, the most reliable indicator of copper excess is the concentration of copper in the liver; however, this is difficult and intrusive to measure in humans (Milne, 1998; Stern et al., 2007).

Acute exposures can lead to tachycardia, respiratory disturbances, and liver and kidney failure (Stern et al., 2007). Incidents of copper toxicity are limited to rare cases of accidental ingestion of contaminated alcoholic beverages (Wyllie, 1957); accidental or deliberate ingestion of high quantities of copper salts; or exposure to drinking water with elevated copper concentrations (IPCS, 1998). Acute toxicity studies have looked at the early effects of excess copper intake before toxicity occurs. The first and most frequent response to elevated levels of copper is low-intensity nausea (Olivares et al., 2001). Other symptoms may include vomiting, diarrhea and adnominal pain (Araya et al., 2001; Pizarro et al., 1999a; Spitalny et al., 1984). These symptoms have been found to resolve when copper is eliminated from drinking water or beverages.

There are three experimental studies that have defined a threshold for acute gastrointestinal upset at 4-5 mg Cu/L of drinking water in healthy adults (Araya et al., 2001, 2003a; Pizarro et al., 1999a, 1999b). Araya et al. (2003) have conducted a multi-site international study to confirm the acute no observed adverse effect level (NOAEL) and the lowest observed adverse effect level (LOAEL) for acute exposures to copper via consumption of bottled drinking water. They have also used the data to develop an exposure-response curve for the incidence of nausea.

1.3C Copper and Chronic Disease

Several studies have looked at the association between serum levels of copper and risk of disease. The cross-sectional nature of some of these studies prevents any direct conclusions from being made with respect to a causal association, but suggests areas for further research. There have also been a series of case-control and cohort studies that provide valuable information on the risk of copper toxicity from excess and deficiency.

A cohort study of 29,368 women aged 55-69 at baseline looked at the association between intake of antioxidant micronutrients including copper and risk of rheumatoid arthritis (Cerhan et al., 2003). Copper intake was assessed in 1986 using a 127-item food frequency questionnaire where subjects were asked how often, on average, over the past year they had consumed each specified food. The subjects were also asked whether they used supplemental copper on a regular basis; however, information was not collected on the dosage, frequency or duration of use. A total of 152 cases of rheumatoid arthritis were identified over the 11 year follow-up period. After controlling for other risk factors, any use of supplemental copper showed a possible inverse association with rheumatoid arthritis (RR = 0.54, 95% CI: 0.28, 1.03) (Cerhan et al., 2003). Relative risks were adjusted for age, total energy intake, marital status, smoking history, age at menopause, use of hormone replacement therapy, decaffeinated coffee consumption, and tea consumption. It is important to note that no information on the duration of supplement use was collected and dietary information was only assessed once at baseline. While copper supplement use was found to decrease ones risk of rheumatoid arthritis, no association was found with dietary copper intake. The investigators comment on the fact that as there was no association between dietary copper and rheumatoid arthritis this complicates the interpretation of the findings as it is not clear whether the inverse association with supplement use is due to

elevated intake through use of supplements, an error in the measurement of micronutrients in the food frequency questionnaire, residual confounding by another health behavior, or a chance finding.

Abnormal copper metabolism has been suggested to play a role in coronary heart disease. Approximately 70 anatomical, chemical and physiological similarities have been found in animals deficient in copper and in people with ischemic heart disease (Klevay, 1993b; 1995; 1998). To examine the association between copper and glycemia, plasma lipids, and atherosclerotic disease, a cross-sectional analysis was conducted on an adult population based cohort (n=1,197) (Bo et al., 2008). A food frequency questionnaire, which assessed mean frequency and portion size for a variety of foods consumed during the 12 months prior to the examination, was used to calculate nutrient intake. Diastolic blood pressure, circulating glucose, uric acid, and low-density lipoprotein (LDL) significantly decreased from the lowest (1.12 mg Cu/day, 0.29 SD) to the highest tertile of copper intake (2.29 mg Cu/day, 1.08 SD) (Bo et al., 2008). This trend did not adjust for other dietary components. Higher tertiles of copper intake were also associated with diets high in fiber, magnesium, vitamin C, beta carotene, and vitamin E. However, the magnitude of the effects did not change after adjusting for age, body mass index (BMI), exercise level, smoking, and dietary intake of total energy, cholesterol, magnesium, zinc, alcohol, and sex. The investigators commented on the fact that due to the cross-sectional nature of the study, even a strong association does not confirm a causal relationship and there could be possible uncontrolled or unknown confounders involved.

Excess copper intake can impact cardiovascular health. In a cross-sectional study, 72 patients with rheumatic vascular heart disease were divided into three subgroups based on the severity of their disease. There were 32 healthy controls. As the severity of rheumatic

heart disease increased, so did serum levels of copper (Kosar et al., 2005). The investigators commented on the fact that the increased serum copper concentration might reflect an ongoing inflammatory process.

In another cross-sectional study the aim was to compare the serum levels of Cu and Zn and the Zn/Cu ratio in 30 patients with ischemic cardiomyopathy and 27 healthy volunteers. Copper levels in patients with ischemic cardiomyopathy were found to be higher than copper levels in healthy controls (Shorkrzadeh, et al., 2009). The results were consistent with Kosar's study (2005) suggesting that copper may have a role in the development of heart disease.

The International Programme on Chemical Safety (1998) reviewed studies published prior to 1998 looking at the association between copper imbalances and cancer. They concluded that the available analytical epidemiological studies in which concentrations of copper in serum were determined only following diagnosis of cancer are uninformative for eludiating a causal association between cancer and copper intake. Serum levels of copper are often elevated in humans with cancer which is likely the result of the body's biological response to cancer occurrence (Fisher et al., 1978). Recently, increased serum levels of copper have been found in patients with leukemia (Zuo et al., 2006); prostate cancer (Ozmen et al., 2006); patients with hepatocellular carcinoma (Lin et al., 2006); cancer of the gastro-intestinal system (Boz et al., 2005); and carcinoma of the gallbladder (Gupta et al., 2005). In a case-control study conducted by Adzersen (2003), 310 cases with primary breast cancer and 353 controls were used to look at the association between the intake of raw vegetables, total vegetables, whole-grain products, selected vitamins and minerals and risk of breast cancer. A food frequency questionnaire was used to collect information on nutrient intake. Four quartiles of copper intake were defined (<2.1, 2.1-2.6, >2.6-3.2 and >3.2 mg

Cu/day). The investigators did not find that copper had a risk reducing effect. The association adjusted for age, total energy intake, age at menarche, age at first birth, age at menopause, mother or sister with breast cancer, smoking status, history of benign breast disease, BMI, alcohol consumption, and hormone replacement therapy. The investigators commented on the limitations of case-control studies including recall and selection bias. Furthermore, as the dietary habits were measured the year before hospital admission they may not necessarily reflect the dietary pattern in the past when the cancer developed.

A case-control study in Burgundy France compared the nutrient intake of 171 colorectal cancer cases and 309 subjects in the general population (Senesse et al., 2004). A food history questionnaire administered by a personal interview was used to determine the mean composition of the diet in macro- and micronutrients including copper. Copper (2.8 mg/day) was associated with an overall increased risk of colorectal cancer compared to controls (0.8 mg Cu/day) (Senesse et al., 2004). Odds ratios did not adjust for other nutrients in the diet. The investigators comment on the fact that as there are high correlations between nutrients and complex biological interactions between micronutrients, attributing an effect to any specific nutrient including copper must always be made with care.

A case control study with 676 incident lung cancer cases and 1,676 healthy controls looked at the association between dietary copper and lung cancer risk (Mahabir et al., 2007). A food frequency questionnaire was used to collect information on dietary copper intake. With increased dietary copper intakes, there was a 41%, 49% and 66% reduced risk for all subjects consuming 0.99-1.22, 1.23-1.56 and >1.56 mg Cu/day respectively compared to subjects consuming <0.99 mg Cu/day (Mahabir et al., 2007). The investigators acknowledge the issues with recall bias and residual confounding and the inherent measurement errors of food frequency questionnaires. The investigators also acknowledged the fact that trace

metals could be proxies for other constituents in the vegetable-fruit group. However, they did recommend that dietary trace metals be considered when lung cancer risks are investigated.

In a prospective study, a total of 34,637 Iowan women free of cancer were mailed a questionnaire in 1986, following which kidney cancer incidence (n=124) was monitored over a 15-year period (Nicodemus et al., 2004). Food intake was measured using a standard food frequency questionnaire. No associations were found between kidney cancer risk and dietary copper intake; however, reported use of copper supplements was associated with a 4.43 fold greater incidence of kidney cancer (95% CI: 1.41-13.92, p=0.01) compared to those who did not use copper supplements (Nicodemus et al., 2004). It is important to note that dietary supplement use was only assessed at baseline and in the sample of subjects there were only 3 events of kidney cancer in copper supplement users. Similar to what was found in Cerhan et al. (2003), where risk of rheumatoid arthritis was associated with copper supplement use but not dietary copper intake, the inverse association with supplement use could be due to elevated intake through use of supplements, errors with the food frequency questionnaire, residual confounding by another health behavior, or a chance finding. At this time, more extensive human studies are required to determine what role copper may play in the etiology, prevention or treatment of cancer.

It is known that copper is an essential component of enzymes involved in brain metabolism. Several neurodegenerative diseases including Alzheimer's disease are characterized by modified copper homeostasis. Modified copper homeostasis may contribute either directly or indirectly to increased oxidative stress and the progression of neurodegenerative diseases. In a recent study, absolute copper (i.e., caeruloplasmin bound copper and free copper in serum) was found to be higher in Alzheimer's disease patients

who were apolipoprotein E4 (APOE4) carriers (a gene associated with late-onset Alzheimer's disease) (Squitti et al., 2007). The fraction of copper unbound to ceruloplasmin has been correlated with cortical delta rhythms across healthy elderly, those with mild cognitive impairments and Alzheimer's disease subjects (Babiloni et al., 2007). In one study on women, serum copper concentration had an inverse linear association with measures of cognitive function (Lam et al., 2008).

In a community based cross-sectional study of 3,718 Chicago residents 65 years and older, dietary intakes of copper and fat were related to changes in global cognitive scores (Morris et al., 2006). Dietary information was assessed with a modified Harvard food frequency questionnaire. An indicator variable was defined to identify persons whose dietary intake of saturated fat was in the top 20% and whose intake of trans fat was in the upper 60%. Overall, dietary intakes of copper, zinc, and iron were not associated with cognitive decline after adjustment for multiple confounders. However, among persons whose diets were high in saturated and trans fats, higher copper intake (2.75 mg Cu/day) was associated with a faster rate of cognitive decline (Morris et al., 2006). It has been suggested that dietary copper may interfere with clearance of amyloid-β from the brain and may further promote amyloid-β accumulation. The investigators acknowledge the limitations of the study design in establishing a causal association and commented on the fact that as the supporting evidence on this association is limited, results must be viewed with caution.

There are limitations in the available epidemiological studies that have found associations between elevated and low intakes of copper and risk of chronic disease. Cohort studies have not been able to incorporate changes in diet over time, and some studies have used small samples or specific subgroups in the population (defined by ethnicity, age, or geographic location) which limits their generalizability (Cerhan et al., 2003). Recall and

selection bias has been clearly recognized as a limitation in all case-control studies that are available (Adzersen et al., 2003; Mahabir et al., 2006; Senesse et al., 2004). Inverse associations between copper intake and cancer risk are limited by the fact that copper is an important constituent of fruits and vegetables and it is possible that copper could be a proxy for other nutrients or phytochemicals in the vegetable-fruit group (Adzersen et al., 2003; Mahabir et al., 2007). Epidemiological studies on copper and chronic disease are not able to support strong conclusions with respect to a causal association between copper and chronic disease (such as arthritis, cancer, or cardiovascular disease), but have highlighted the fact that essential metallic elements, including copper, need more attention with respect to their roles in the etiology of chronic disease.

1.4 POPULATIONS AT INCREASED RISK

Much of what is known about copper homeostasis comes from studies on humans with inborn errors of copper metabolism (IPCS, 1998). Individuals with Menkes Disease, an X-linked genetic syndrome, are severely copper deficient, due to the inability of copper to be pumped out of intestinal cells for transport into the blood. This leads to abnormal growth and development and severe deterioration of the nervous system (Kaler, 1994; 1996; 1998). Individuals with Menkes disease rarely survive past early childhood (IPCS, 1998). Wilson's disease is an autosomal recessive inherited disorder of copper transport, in which copper accumulates in various organs in the body which can cause neurological or psychiatric symptoms and liver disease (Gitlin, 2003). Indian childhood cirrhosis is a rare condition characterized by unusual liver damage due to a genetic defect in copper metabolism in combination with a high environmental exposure to copper (Wijmenga, 1998). Individuals with liver disease are also at increased risk of toxicity from several nutrients and minerals including copper.

Susceptibility to excess levels of copper will depend on species, genetics, age, and diet (Fuentealba et al., 2000). Variation in risk is partly due to differences in efficiency of absorption and excretion; intake of other hepatoxic or protective factors; cellular distribution of copper; and the expression of copper transport and storage proteins (Fuentealba et al., 2000).

Variation in exposure with time and life stage is an important determinant of risk. Copper is essential for intrauterine growth and development of the fetus during the third trimester when copper needs to accumulate and be stored for the immediate post-delivery period (Yip & Dallmnan, 1996; IPCS, 1998). Healthy newborns are protected from copper deficiency as a considerable amount of copper is transferred from the mother to the fetus by the end of the gestation period and a large proportion of the accumulated copper is retained in the liver. Premature infants may be at increased risk of copper deficiency as a shortened gestation period or premature birth can result in inadequate accumulation of copper stores (IPCS, 1998).

Varada et al. (1993) examined changes in the kinetic characteristics of copper absorption at three stages of rat development (i.e., suckling, weanling and adolescence). They found that during the suckling period and at weaning, when rats transition to solid foods, rats will absorb copper in a concentration-dependent fashion; however, there appears to be no feedback control or saturability for copper transport during the third week of life (Varada et al., 1993). In adolescence, the overall copper absorption capacity of rats declines and saturability of a mediated component of copper intestinal absorption develops (Varada et al., 1993).

For copper excess, some animal studies have shown that males are more sensitive to copper toxicity, whereas in other studies females appear to be more sensitive. Shiraishi et al

(1993) reported that male mice 8-9 weeks of age were more sensitive to copper induced toxicity than females; no effect of sex was found among younger mice. Male Fisher 344 rats (7-14 weeks of age) injected intravenously with copper accumulated more copper in the liver than female rats (Nederbragt, 1985). While a statistically significant difference between males and females was not seen in a study of copper induced toxicity, 2 of the 8 Cu-loaded female rats died during the experiment, and female rats accumulated almost 100 ppm more Cu than males (Fuentealba et al., 2000). In another study conducted by Linder et al. (1979), Cu-induced hemolysis was observed in 2 of the 3 female rat strains, whereas no male rats developed hemolysis (Linder et al., 1979). Bremner et al. (1981) reported that renal copper concentrations were twice as high in female Hooded Lister rats (7-9 weeks) compared to males of the same age (1981).

As with copper excess, the evidence is mixed on whether males or females are more sensitive to states of copper deficiency. Bureau et al. (2003) found that female rats had a greater degree of protection against oxidative damage than male rats, and their anemia was less severe (Bureau et al., 2003). There may be biological reasons for why females are less sensitive to copper deficiency. There is evidence that estrogen may have protective effects against copper toxicity through increased antioxidant status (Bureau et al., 2003). There are conflicting reports in the literature suggesting that females may not always be less sensitive to copper deficiency. Female rats were found to be more susceptible to cardiac hypertrophy, anemia and decreased body weight following a deficient copper diet (Farquharson et al., 1988). The severity of these effects were found to be less in males (Farquharson, et al., 1988). It has been noted that the apparent resistance of female rats to states of copper deficiency may not be maintained to the same degree when challenged by very severe copper deficiency (Bureau et al., 2003).

There have been a few studies on humans looking at differences between males and females in terms of the homeostatic mechanisms that regulate internal levels of copper. There is limited data on differential rates of copper absorption or excretion in men and women. Plasma copper and ceruloplasmin have been found to be higher in women than men (Mason, 1979; Milne et al., 1988; Milne et al., 1990). Variation in the levels of biomarkers of copper status between males and females are likely a result of differences in circulating concentrations of estrogen (Mason, 1979, Solomons, 1979, Evans et al., 1970). In a study by Johnson et al. (1992), they examined the effect of sex on copper absorption, biological half-life and status in humans using a standard diet that was extrinsically labeled with 92.5 kBq ^{67}Cu. Women (20-59 years of age) absorbed more copper than men from the labeled diet and had more rapid turnover of copper after absorption. However, when copper intake was standardized by body weight, the differences between men and women disappeared. The authors concluded that on a body-weight basis, men and women have similar copper requirements; however, in terms of total dietary intakes, women have smaller copper requirements than do men (Johnson et al., 1992).

1.5 COPPER HOMEOSTASIS

The body has a complex regulatory system to maintain internal concentrations of copper within an appropriate range through coordination of copper uptake, distribution, metabolism, and excretion. The homeostatic range for copper is determined by a well-coordinated series of adaptive responses including changes in gastrointestinal absorption and/or billiary excretion, activation or inactivation of multiple binding sites, alterations in transport mechanisms and hepatic storage, and release. The amount of copper in the intestinal lumen, the ratio between promoters and inhibitors of absorption, and copper nutritional status, all influence copper homeostasis (Araya et al., 2003c). The intestine has

the primary responsibility for maintaining copper homeostasis. Absorption can range from 15-97% depending on copper content and dietary composition (Stern et al., 2007; Strickland et al., 1972a, 1972b; Turnlund et al., 1989; Turnlund, 1998; Ehrenkranz et al., 1989). Copper can interact with several other nutrients including other essential trace elements. For example, high intakes of zinc can inhibit the absorption of copper (WHO, 2002). Iron, molybdenum, lead, and cadmium can also influence dietary copper absorption (Cousins, 1985; Oestreicher & Cousins, 1985). Bioavailability of copper can also be reduced by carbohydrates, dietary cellulose fiber, and phytates (Lee et al., 1984; Greger et al., 1985; Werman et al., 1995; Wapnir, 1998). Since copper is only partially absorbed in the duodenum of the gastrointestinal track, the amount consumed does not equal the amount absorbed (Wapnir, 1998). The majority of copper absorption occurs in the small intestine; however, some copper will be absorbed in the stomach where the acidic environment can promote copper solubility by dissociating copper from macromolecules (Harris, 1997; Turnlund, 1999). During absorption, excess copper is sequestered in enterocyte metallothioneins (Stern et al., 2007). Once copper has entered intestinal cells, it then moves into serosal capillaries, binding to albumin, glutathione, amino acids, and transcuprein (Marceau et al., 1970; Bligh et al., 1992; Linder et al., 1996). Copper eventually enters the liver, where it can be transported to extra-hepatic tissues or excreted in the bile (Ralph & McArdle, 2001). While the majority of copper in the body is found in bone and muscle, the liver is the key site for regulating plasma copper concentrations (Olivares & Uauy, 1996; Turnlund, et al., 1998) and the bile is the primary pathway by which copper is excreted.

1.6 TYPICAL EXPOSURES AND NUTRITIONAL REFERENCE VALUES FOR COPPER

In 2008, Cockell and associates reviewed the regulatory frameworks for chronic copper exposure. The third National Health and Nutrition Examination Survey (NHANES

III) in the United States found that the estimated mean copper intake from food and supplements was 1.50 mg/day for the general population including pregnant and lactating women; 1.54 to 1.70 mg/day for men, and 1.13 to 1.18 for women (Food and Nutrition Board, 2001). They also reported that approximately 15% of adults in the United States consume supplements containing copper (Food and Nutrition Board, 2001). While food accounts for the majority of one's daily copper intake, drinking water can also provide a significant source especially if there is high dissolution from copper pipes. A survey in Ontario, Canada found that drinking water contained an average of 0.18 mg Cu/L (Health Canada, 2008).

In 2001, the U.S. National Academy of Sciences' Food and Nutrition Board decided that data on copper excess available from copper depletion-repletion studies was adequate to define a recommended dietary intake (RDI) for copper (Food and Nutrition Board, 2001). For adult men and women the RDI is currently set at 0.9 mg Cu/day (Food and Nutrition Board, 2001). The RDI is defined as being equal to the estimated average requirement (EAR) plus twice the coefficient of variation (set at 15%) to cover the needs of 98% percent of individuals (the RDI is thus 130% of the EAR). In North America the EAR is the intake level for a nutrient at which the needs of 50% of the population will be met (Cockell et al., 2008). No single indicator was judged to be adequate for deriving the EAR for adults. In order to set the EAR for copper, a combination of indicators from controlled depletion-repletion studies were used including plasma copper, ceruloplasmin, erythrocyte superoxide dismutase activity and platelet copper concentration (Food and Nutrition Board, 2001). The Food and Nutrition Board comments on the fact that variation in these indicators do not always reflect dietary intake and may not be sensitive enough to detect marginal copper status (Food and Nutrition Board, 2001). Furthermore, traditional indicators of copper

status including serum copper and ceruloplasmin also increase during pregnancy and in a number of diseases (Food and Nutrition Board, 2001). Data from three studies were used to set the EAR at 0.7 mg Cu/day (Turnlund et al., 1990; Milne et al., 1996; Turnlund et al., 1997).

The U.S. Food and Nutrition Board (2001) have prescribed an upper safe limit of 10 mg Cu/day. The upper safe limit was based largely on a double-blind supplement study showing normal liver function in adults consuming 10 mg Cu/day. In North America an uncertainty factor of 1 is used because the NOAEL is considered safe for most of the population. However, in Europe the upper safe limit is set at 5 mg Cu/day (Cockell et al., 2008), based on an uncertainty factor of 2 to account for the potential variability in a normal population. It is important to note that the upper safe limit is only based on liver toxicity endpoints, and does not take into consideration less severe but potentially clinically important responses.

1.7 CHALLENGES IN COPPER EXPOSURE-RESPONSE ASSESSMENT

As copper can lead to both toxicity from excess and deficiency, it poses a challenge to the risk assessment process. Exposure-response relationships provide the foundation for setting recommended levels of exposure for essential and non-essential substances. As the slope of the exposure-response curve has not yet been characterized for copper and may differ in magnitude between deficiency and excess, there remains some uncertainty with respect to what levels should be recommended to balance the risk of toxicity from both excess and deficiency (Food and Nutrition Board, 2001). Traditional approaches used to set safe limits for chemical exposures have used large uncertainty factors; however, the application of the uncertainty factors to essential elements can result in recommendations that are nutritionally inadequate. In order to fully understand the implications of copper

toxicity on human health and implement sound management strategies, the exposure-response relationship for copper needs to be adequately defined.

The assessment of risk from essential metallic elements is an issue for both toxicologists and nutritionists; however, the development of appropriate risk assessment methodologies in these two fields has not been synchronized. There is a need for a common approach to assess the risks of adverse effects from copper excess and copper deficiency. A report prepared by the WHO entitled "Principles and Methods for the Assessment of Risk from Essential Trace Elements," highlights an issue that is applicable to the risk assessment process for copper where the margin between the lower and upper limit of the acceptable range of oral intake many be very small, and in some cases these values may actually overlap among individuals and populations (WHO, 2002). Overlap between the lower and upper limits of the acceptable range of oral intake (AROI) can be due to several factors including different methodologies in nutrition and toxicology, minimal coordination between these two fields, and lack of coordination between different advisory groups and regulatory agencies.

Table 1.2 presents a comparison of the principles and methods for regulatory decisions in toxicology and nutrition (WHO, 2002). Regulatory guidelines in nutrition are defined for both food and water. In toxicology, the tolerable upper intake level is defined for all sources of exposure; however, the reference dose is only defined for oral exposures. In nutrition, data used to set regulatory guidelines relies on a combination of studies on nutrient and dietary interactions, whereas in toxicology only toxicity data is considered. Guidelines in nutrition are usually set for specific age and sex groups as well as subpopulations with different physiological states. In toxicology, all healthy population groups over three months of age are usually considered.

Table 1.2: Principles and Methods for Regulatory Decisions in Toxicology and Nutrition

Principle	*Nutrient Requirements*	*Toxicological Limits*
Human exposure	Food and water	All sources for tolerable upper intake level (TI), oral sources only for reference dose (RfD)
Use of data	Bioavailability, nutrient and dietary interactions all considered	Only toxicity is usually considered
Population addressed and protected	Usually developed for specific age-sex groups, physiological states	Usually all healthy groups of consumers over three months of age
Clinical significance	Deficiency states can lead to clinical effects or inadequate stores	Adverse end-point chosen often with limited information on clinical significance.

Adapted from the WHO, 2002

Figure 1.1 provides a theoretical representation of the AROI for essential trace elements including copper (WHO, 2002). The AROI or the homeostatic range is represented as a trough in the U-shaped exposure-response curve between points A and B in Figure 1.1. Neither the lower or upper limits of the AROI are absolute values, with no risk of an adverse effect occurring. Variability between individuals, characterized by the 'distribution of risk of toxicity' and 'distribution of requirements' in Figure 1.1, can be due to differences in homeostasis, bioavailability, age-related factors, and dietary and nutrient interactions.

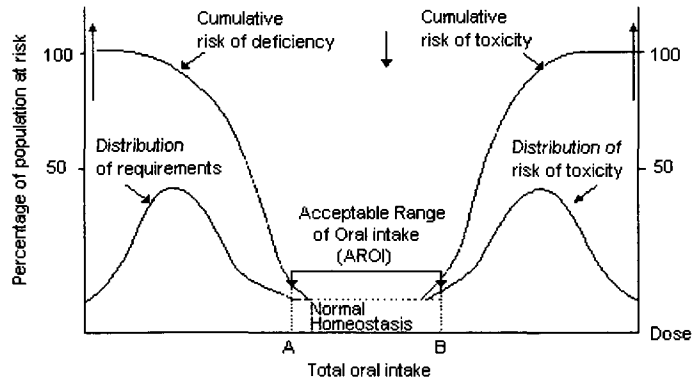


Figure 1.1 Theoretical Representation of the Acceptable Range of Oral Intake (WHO, 2002). Reprinted with permission from the World Health Organization.

Figure 1.2 presents another theoretical representation of the acceptable range of oral intake, wherein more than one exposure-response curve is defined for four levels of severity including death; clinical effects; subclinical biomarkers of effect with functional impairments; and bioclinical markers without functional significance (WHO, 2002).

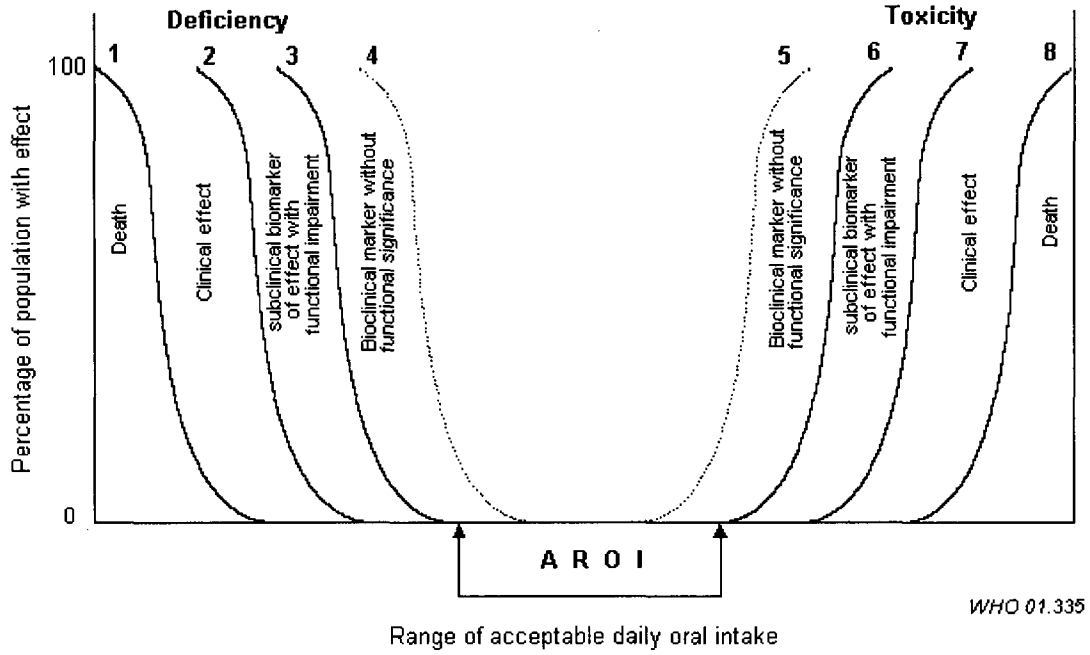


Figure 1.2 Theoretical Exposure-response Curves for Various Effect Levels (WHO, 2002). Reprinted with permission from the World Health Organization.

There are several challenges in defining the zone of homeostasis, the margins of protection outside the normal homeostatic range, and the exposure-response curves which are likely to differ in magnitude between deficiency and excess. There remain several uncertainties at the molecular level regarding the transport of copper across human and animal tissues which limits the application of biological based exposure-response models for copper. Furthermore, the pharmacodynamics of copper excess or deficiency have yet to be adequately described. Empirically-based approaches could be applied at this point to integrate the diverse range of data.

1.8 BACKGROUND ON CATEGORICAL REGRESSION

A recent review in the *Journal of Toxicology and Environmental Health* described possible exposure-response modeling strategies for copper (Stern et al., 2007). Traditional risk assessment approaches involve identifying the most scientifically sound NOAEL from experimental animal studies and then dividing the NOAEL by an uncertainty factor to account for inter-species differences in sensitivity (a default value of 10 is typically used in the absence of information to the contrary) along with an additional factor (usually 10) to allow for interindividual variability in susceptibility within the human population. Additional uncertainty factors may be used to allow for less than lifetime studies, less than optimal data, or other attributes of the available data (Krewski et al. 1991). When a NOAEL is unavailable, the lowest observed adverse effect level (LOAEL) is often used in place of the NOAEL, along with the use of an additional uncertainty factor (often 5-fold) to account for the absence of a NOAEL. The NOAEL or LOAEL approach is a simple and familiar method in risk assessment, using a single exposure-time data point that is selected based on the presence or absence of effect. This approach makes limited use of the available exposure-response information and does not provide any direct information on the uncertainty of the estimate. Benchmark dose (BMD) modeling is an example of one of the more sophisticated approaches that have been developed for dose-response assessment. The benchmark dose is a modeled point in the dose-response curve of an adverse effect corresponding to a predetermined increase in risk (in the range of 5-10%, adjusted for background response) when compared to controls (Stern et al., 2007). This is an empirical curve-fitting strategy that uses all the dose-response data at one time point and is able to demonstrate uncertainty in the estimate. Benchmark dose modeling cannot, however, take into account several adverse health effects that may occur simultaneously.

It is important to note that BMD modeling is relevant to both empirical and biologically based dose-response models (Krewski et al. 2002). Biological based dose-response modeling quantifies biological mechanisms to determine the toxic effects of chemical agents and in the future, may serve as a potential alternative to the use of laboratory experiments. Biologically based dose-response models are of particular interest in the risk assessment of essential metallic elements, since different mechanisms may lead to adverse health outcomes from both states of excess and deficiency. While research is advancing on the homeostatic properties of copper, there is insufficient information available on the molecular mechanisms, pharmacodynamics and mechanisms disrupting homeostasis to define a biologically based exposure-response model for copper.

Hertzberg and Dourson (1993) identified statistical regression using ordered categories of overall toxicity as a superior method for non-cancer risk assessment. In the review by Stern et al. (2007), several potential modeling approaches were considered based on the available data on copper excess and deficiency. They commented that:

"While a mechanistically based approach to dose-response modeling provides a useful concept for organizing and integrating the large and diverse database for copper toxicity, the limitations of the toxicity database at the present time likely impede the successful implementation of such an approach. Most studies in the database are descriptive rather than mechanistic in nature. Most studies are limited in terms of experimental design, with only one or two nonzero dose groups, and observations recorded at a single time point, in a single species, representing toxicity in a single organ system arising from either copper excess or copper deficiency, but rarely both. There is insufficient information available on any endpoint, as assayed in any single study, to produce a well-defined dose-response curve for both copper deficiency and excess. Thus, there is a need to consider empirical approaches to modeling multiple studies and endpoints simultaneously using some form of common toxicity metric."

Ordinal regression involves the organization of response data in the form of ordered categories of severity and the application of regression analysis to predict the probability of achieving a particular severity category as a function of one or more independent variables (such as duration and level of exposure). Considerable attention and professional judgment

is required to define the ordinal severity scale, where endpoints extracted from studies on both copper deficiency and/or excess are assigned to a single category ranging from a low to a high severity level (e.g. 0= no observed effect level, 1= no observed adverse effect level, 2 = adverse effect level and 3 = severe effect level). These numerical assignments (0, 1, 2, and 3) are not used directly in the calculations, and only indicate the ordering of the severity categories (Hertzberg and Dourson, 1993). When there are multiple responses for each level of exposure, severity scores can be assigned to each response and the highest severity score can be selected to represent the exposure group.

When analyses are extended beyond dichotomous variables to the analysis of categorical dependent variables with more than two categories, the resulting analyses have been termed polytomous logistic regression, ordinal regression and categorical regression. The term categorical regression, referred to by the United States Environmental Protection Agency (US EPA), will be used to describe the proposed exposure-response analyses in this thesis. Categorical regression analysis can be implemented using CatReg, a software package that runs in R. CatReg was developed by the United States Environmental Protection Agency's National Center for Environmental Assessment and was designed for toxicologists and health scientists to conduct exposure-response analyses. Several articles in health risk assessment emerged between the mid 80s and mid 90s discussing potential methods for modeling effects of graded severity, of incorporating data from diverse sources, and analyzing ordinal response data. In 1996, one of the key methods papers behind the development of CatReg was published by Simpson and associates (Simpson et al., 1996). This paper provides a detailed description of methodology developed for regression analysis of ordinal response data subject to interval censoring. To address the potential for within

study correlations, the authors developed a generalized estimating approach to the problem, with appropriate adjustments to uncertainty statements.

Special features offered by CatReg include: stratifying the analysis by user-specified covariates; choosing among several basic forms of the exposure-response curve; using effects assigned to a range of severity categories, rather than a single category; the use of cluster-correlated data; use of user-specified weights; and options for filtering sections of the data for sensitivity analyses (US EPA, 2000). Two basic models are available in the program including the cumulative odds model and the unrestricted cumulative model. The differences between these two models will be covered in section 3.6. The user can decide whether either model conforms to the logistic, normal or Gumbel cumulative probability distribution. The program uses link functions to transform the probability for each severity level to a linear function of the unknown parameters (US EPA, 2000). The program also has several options for testing model parameters, conducting sensitivity analyses, and generating plots of the raw data and fitted models.

1.9 PROJECT RATIONALE

Due to the complex nature of essential metallic elements and the limitations of traditional modeling approaches, categorical regression could be considered as the most appropriate risk assessment tool to define the limits of the acceptable range of oral intake (Stern et al., 2007). Most of the data on copper excess and deficiency report responses with descriptive or continuous data for an entire exposure group. Another issue with the data on copper excess and deficiency is that in individual experiments, a limited range of possible human exposure durations and concentrations have been utilized. Categorical regression allows several studies to be combined so that benchmark toxicity values can be identified for a larger range of exposures and durations. In the categorical regression analysis, a more

comprehensive database can be used for the analysis as continuous, descriptive, categorical, and incidence data can be defined by a common severity category. Unlike other meta-analysis procedures, categorical regression can define the exposure-response relationship with respect to increasing severity of response. It has been argued that the use of categorical regression may be more appropriate than BMD modeling for low dose extrapolations as categorical regression can be used to combine data from multiple studies and utilize information on multiple animal species (Haber et al., 2001). As the exposure-response curve is no longer based solely on the most sensitive strain, animal species or sex it may be more predictive of actual human risk. Furthermore, the scatter about the fitted regression model provides useful information on the uncertainty in the exposure-response model.

At this time, rough estimates have been used to set national and international recommendations for copper. A workshop on the risk assessment of trace essential elements identified the need to improve coordination between nutrition and toxicology to set acceptable ranges of oral intake for trace elements including copper (Goldhaber, 2003). Variability in the recommended values for copper exposure has been found to be primarily due to differences in the use of uncertainty factors and data sets (Goldhaber, 2003). A review of the science behind the current drinking water standard was published in 2001 and concluded that regulatory standards for copper, including the European Union's drinking water directive and the World Health Organization's recommendations, do not have a firm scientific basis (Fewtrell, et al., 2001). This report recommended clear and transparent reporting of the evidence that is used to support exposure standards. The WHO guideline on copper levels in drinking water has gone through a series of changes to accommodate new evidence on copper toxicity (Fewtrell et al., 2001). This guideline explicitly states that there is uncertainty regarding the long-term effects of copper in potentially sensitive human

populations and that there is some evidence from studies on experimental animals that chronic exposure to high levels of copper may be associated with neurological effects (WHO, 2004). In the absence of comprehensive information to support a risk assessment for copper, regulatory bodies have set recommendations based on the available scientific evidence or aesthetic factors. For example, in Canada, an aesthetic objective has been set for copper levels in drinking water to avoid laundry stains and to ensure that the water does not have a metallic taste (Health Canada, 1992).

In May 2008, a workshop held in Ottawa provided an opportunity to present and address the limitations of modeling the exposure-response relationship of essential metallic elements including copper and discuss the potential utility of categorical regression methods for exposure-response assessment. Valuable insight was gained from experts from Canada, the United Kingdom and the United States.

As copper has important and diverse commercial applications, the copper industry is encouraging research to ensure that regulatory decisions are based on sound scientific knowledge. The lack of information on copper toxicity and deficiency has led regulatory agencies to use precautionary approaches to develop occupational and environmental exposure guidelines for copper. There is concern from an industry perspective that regulatory decisions based on alleged harmful effects of copper will negatively impact the copper industry and will slow down research on innovative applications of copper. As a result, the copper industry, including the International Copper Association encourages and values furthering the knowledge base in this area. The general public, industry and national and international regulatory bodies could potentially benefit from having a better understanding of the relationship between copper excess and deficiency.

Establishing safe limits for essential elements is currently an active area of research. At present, there remain several uncertainties with respect to how different approaches can be applied to adequately define the limits of the acceptable range of oral intake. As the application of categorical regression has not been applied to define the exposure-response relationship for other essential metallic elements, this project is essentially testing out an innovative approach that could be replicated for other essential metallic elements such as zinc and manganese. Categorical regression will make better use of current research efforts by applying an approach that effectively integrates multiple studies into a single quantitative analysis. This process will also help identify areas in need of additional data to refine the exposure-response relationship for copper excess and deficiency.

PART 2: AIMS AND OBJECTIVES

The overall aim of this thesis is to:

- characterize the exposure-response relationship for copper excess and deficiency.

The specific objectives are to:

- define the limits of the acceptable range of oral intake for chronic exposures at different risk probabilities;
- evaluate the impact of incorporating acute exposure studies in a combined analysis with subacute, subchronic and chronic exposure studies;
- evaluate the impact of the animal species, age, sex, route of exposure (drinking water versus feed), and solubility on the risk of adverse effects from copper excess and deficiency;
- determine the magnitude of the differences between ERC10 estimates between animal species after the adjustment for body weight or surface area;
- identify limitations in the application of categorical regression to characterize the exposure-response relationship for copper and other essential metallic elements; and
- identify information gaps in the literature that could improve the precision of the estimates around the acceptable range of oral intake.

While there are two major routes of exposure to copper (inhalation and oral exposures), the focus of the current copper database is on studies looking at the health effects from oral exposures.

PART 3: METHODS

Exposure-response data from studies identified in a literature review have been assigned to severity scores and integrated into a copper toxicity database. This database was used to conduct a categorical regression analysis. There were several steps involved in building the categorical regression model so that it adequately described the data available in the copper toxicity database. The models that were defined in the initial series of analyses were used to characterize the exposure-response relationship for copper excess and deficiency, define the limits of the acceptable range of oral intake and evaluate the magnitude of the difference between the extra risk concentration (ERCq) for different animal species. The process used to build the categorical regression model allowed us to evaluate the impact of acute exposure studies and the impact of different variables (e.g., animal species, routes of exposure, sex, age) on the severity of response. The first section (3.1) in Part 3 will describe previous work by a group of toxicologists and health scientists to identify studies for the copper toxicity database and assign severity scores. Sections 3.2 to 3.6 will describe: how the data in the copper database was modified to create a common dose metric; how the copper database was reviewed prior to the categorical regression analysis; how the exposure-response data was defined; and the software that was used to conduct the categorical regression analysis. Sections 3.7 and 3.8 will discuss the strategies used to account for group size and cluster samples. The final sections will describe the following: how the categorical regression model was defined in terms of the stratification and transformation options; how sensitivity analyses were used to evaluate the effect of different variables in the regression analysis; the strategies used to test assumptions and evaluate the model fit; and finally, how the final estimates were used to inform the limits of the acceptable range of oral intake.

3.1 COPPER DATABASE

Section 3.1 describes what has already been done by a group of experts in toxicology and health risk assessment to build a copper exposure-response database. Additional information on the copper exposure-response database can be found in Stern et al. (2007). In 2002, the literature on copper toxicity from excess and deficiency was reviewed to assess whether there was sufficient information available to define the exposure-response relationship for copper. The search strategy was developed by a librarian scientist from Copper Research Information Flow. The literature search identifies citations relevant to copper toxicity and deficiency where the focus is on terrestrial organisms and humans. The search strategy is designed to identify case studies, experimental studies, human health risk assessments, epidemiological studies, and occupational exposure studies.

Once the collection of studies dealing with copper deficiency and excess were obtained, a qualitative binning exercise was conducted, in which a utility score was assigned to each paper. Five utility scores were created, as outlined in Table 3.1. The highest score represented no utility for the exposure-response and the lowest score represented studies with multiple doses or outcomes, adequate reporting and physiological measures.

Table 3.1 Utility Evaluation Framework

Most Useful →				**Least Useful**
1	*2*	*3*	*4*	*5*
Multiple dose or multiple outcomes from intact animals or humans	Multiple or single dose from intact animals or humans	Single dose or clinical study / case report with indeterminate dose	No dose information	No utility
Adequate reporting	Fairly good reporting	Single dose tracer or pharmacokinetic study	Physiological information	
Physiological measures	Likely to yield useful information	Info re. body burden or kinetics	Review	
	Change in time points	Mechanistic or cellular effects		
	Cellular effects			

This preliminary scoring system was developed in order to identify not only the overall quality of the data but also whether the data could be used in an exposure-response assessment. During the original scoring exercise in 2002, over 500 papers were reviewed and assigned a "utility score". The literature search was updated in 2008 using the same search strategy and screening process, following which 341 additional papers were screened and given a utility score.

The second phase of binning involved looking more closely at each paper that had a utility score of 1 or 2. The scoring group developed a common list of quality considerations for human and animal studies as well as a list of exclusion criteria. Quality considerations for humans and animal studies and the exclusion criteria are listed below.

Quality Considerations for Human Studies

1. The study included multiple endpoints.
2. Copper balance studies provided adequate repletion following the period of copper depletion.
3. Controlled clinical study environment or design is optimal; however, other study designs may be adequate.
4. Accurate estimates of copper intake were available.
5. Data were subject to adequate statistical analyses.
6. Separate analyses have been conducted for infants, children and adults.

Quality Considerations for Animal Studies

1. The animal species and stain was considered to be a suitable model for the purpose of human health risk assessment.
2. The route of exposure was relevant to human health risk assessment.
3. Standard considerations for animal study design and performance were applied.
4. In the case of dietary exposure studies, pair feeding designs are optimal; however, other study designs may also be appropriate.
5. The data was subject to appropriate statistical analyses.
6. Separate analyses were conducted based on the age of the animals in the study.

Exclusion Criteria

1. There is inadequate information to characterize the dose and duration of exposure.
2. The information could not be entirely attributed to the effects of copper alone (confounders).
3. Copper was considered as the outcome and not the intervention.

4. Animals or humans have features suggestive of disturbed copper metabolism (transgenic animals, humans with genetic disease, or dietary copper deficiency).
5. The exposure route was not relevant for humans.
6. The animal model is not suitable for human health risk assessment (e.g., ruminant species, invertebrate species).
7. There was inadequate statistical reporting of the data.

It is important to note that while the original scoring team included toxicologists, health scientists and biochemists; epidemiological studies were viewed favorably and considered for inclusion. While several human studies were included in the database including experimental studies and case studies, no cross sectional, cohort or case control studies could be integrated at this time. There were specific issues with each study; however, there were common issues among all the epidemiological studies considered in the review. In epidemiological studies, the average amount of copper in each subject's diet is often estimated from dietary surveys. Due to the varied intake levels in the population, dose has to be defined in tertiles. The aim of exposure-response assessment is to define the level of intake as precisely as possible; however, even in experimental studies on animals and humans, the level of copper intake is not always exact. The median level of copper intake in each tertile could be used to define each dose group. Duration is often a bigger issue in characterizing the level of exposure. In cross-sectional studies we cannot establish a temporal sequence between exposure and disease. In cohort studies such as the kidney cancer study by Nicodemus et al. (2004), dietary information was only collected at baseline. In order to associate levels of copper intake with kidney cancer, one had to assume that the diets of each subject were relatively constant over the 15 year follow-up period. In case-control studies, dietary history is also often collected at one time point and it can be difficult to establish a clear temporal sequence between exposure and disease. Another important issue is the fact that the epidemiological studies available are more oriented towards hazard identification. There is often an interest in a single outcome, for example, risk of lung cancer,

heart disease, or kidney cancer. In order to define an exposure-response curve for copper, information is needed at levels of intake associated with early disruptions of copper homeostasis as well as reversible and irreversible gross toxicity. While a deficient or elevated tertile of copper intake may not be associated with a statistically significance increase in cancer or heart disease, there may be other underlying disruptions in copper homeostasis that are not being measured. The most important issue with the current selection of epidemiological studies centers on establishing a casual association between the exposure and outcome. While associations may be found between elevated copper intake and adverse effects (e.g., chronic disease incidence, clinical characteristics) final risk estimates do not often adjust for other nutrients in the diet. Diets high in copper are also more likely to be higher in other components including antioxidants and vitamins. For example, in Bo et al. (2008) subjects in the highest tertile of copper intake also had elevated levels of fiber, magnesium, vitamin C, beta carotene, and vitamin E in their diet compared to the lowest tertile of copper intake. Epidemiological studies will continue to be considered in further literature review updates as these studies could provide valuable information on chronic exposures to elevated or deficient copper intake in humans.

After the utility scoring process, a second binning exercise was undertaken, in which outcomes reported in each study were assigned a severity score. Severity scores were assigned by isolating each independent endpoint and assigning a single severity score to that outcome. The refinement of the severity levels for copper was guided by a detailed review of indicators of toxicity from excess and deficiency (Stern et al., 2007). A response matrix was created based on this review and was used as a guide for the assignment of severity scores (Table 3.2). The lowest severity score (severity level 0) corresponded with responses at a particular dose level where there was no change compared to controls (i.e., no observed

effect level). Severity level 1 corresponded to homeostatic responses associated primarily with changes in measures of copper or biological systems associated directly with copper metabolism (i.e., no observed adverse effect level). Severity level 2 corresponded with early biological indicators of accumulated copper. Severity level 3 corresponded to metabolic derangements of metabolic substrates that are influenced by copper metabolism (direct or indirect mechanisms). Severity level 4 corresponded to changes that could be described as gross reversible toxic effects, whereas severity level 5 corresponded to irreversible gross toxic effects. Death was given its own category, severity level 6.

Once the severity scores were assigned to each endpoint, a database was designed to record detailed information on the study, including: the abstract, reference identification number, type of study, copper specie, route of exposure, animal specie and animal strain. For each study, the concentration and duration of exposure was defined and all the health endpoints measured were listed, along with an indication of whether the original investigators found a statistically significant change compared to controls.

Table 3.2 Seven-Level Severity Response Matrix

← ← ←Deficiency					Homeostasis	← No Effect →		Homeostasis	Excess → → →				
6	*5*	*4*	*3*	*2*	*1*	*0*	*0*	*1*	*2*	*3*	*4*	*5*	*6*
Death	**Serious irreversible gross deficiency**	**Reversible gross deficiency**	**Metabolic perturbation**	**Early biological indicators of deficient levels of copper**	**Homeostatic adaptations to low intakes**			**Homeostatic adaptations to high intakes**	**Early biological indicators of accumulated copper**	**Metabolic perturbation**	**Reversible gross excess**	**Serious irreversible gross excess**	**Death**
		Gross threatening disturbances of metabolism; disturbances of peripheral products; depletion of liver Cu stores	Metabolic perturbation or change in metabolism of other metals (e.g. Fe); altered immune function; cardiac hypertrophy; membrane fluidity changes; anemia	Loss of Cu-dependent enzyme function especially in tissues with rapid turnover (GI, mucosa); changes in blood cell number or function; altered SOD activity	Use of endogenous Cu stores; decreased Cu excretion; increased GI Cu acquisition			Increased Cu excretion; decreased GI Cu absorption; increased Cu deposition in liver	Changes in Cu dependent enzyme function; changes in Cu absorption & transport; changes in cholesterol and triglyceride levels in blood and liver; large increases in liver Cu burden	Increased GI metallothionein levels, kidney droplets; increased systolic blood pressure; decreased weight gain	Gross dysfunction, disturbances in metabolism of other nutrients; gross changes in morphology		

Tables A1 and A2 in Appendix A present relevant information extracted from the studies in the copper exposure-response database. These two tables present the information on copper toxicity from excess (A1) and deficiency (A2) separately. Each line in the table corresponds to a data point which is represented by a separate severity score and treated as an individual entry in the analysis. The copper database lists the assigned severity scores for all measures in each experiment. As most studies reported multiple responses to copper excess or deficiency, several severity scores might be associated with one exposure level; therefore, a single severity score that corresponds to the most severe effect was selected to represent that exposure group. The practice of selecting the most severe effect (with the highest severity score) was used by Guth et al. (2007) in a previous application of categorical regression to model the exposure-response relationship of tetrachoroethylene.

For each data point, information is provided on the corresponding type of exposure (i.e. acute, subacute, subchronic, or chronic); copper specie (e.g., $CuSO_4$) route of exposure (i.e., drinking water, capsule or diet); animal species (humans, rats, mice, pigs or rabbits); strain; life stage (i.e., adult or weanling); and sex (Table A1 and A2). The tables also list the number of experiments within a study and the number of exposure groups within that experiment.

3.2 COMMON EXPOSURE METRIC

The doses and durations of exposure for each study in the copper toxicity database were converted to a common exposure metric where duration was defined in days and concentration was defined by the amount of copper consumed daily in milligrams (mg), mg/kg body weight (bw), mg/kg $bw^{1/4}$, mg/kg $bw^{2/3}$ and mg/kg $bw^{3/4}$. In the majority of human studies, when the subjects are given diets with elevated or deficient levels of copper, sufficient information is provided in the study on the average amount of copper consumed daily and the average body weight of the study subjects. There are cases where copper is

administered as a capsule and information on background levels of copper in the diet are not provided. Information on the average amount of copper in a typical human diet was estimated from one of the studies in the copper toxicity database that measured habitual dietary intake (Baker et al., 1999a). Information on average feed and water consumption as well as average body weight based on the strain and age of the animal species was estimated from laboratory guidelines for experimental animal studies (Hertzberg, 1989). A systematic process was developed to ensure that any assumptions used to estimate feed or water consumption levels or body weights were documented and standardized across studies.

The estimated weight at the mid-point of the experiment was used to calculate the exposure per kilogram body weight per day for all species other than humans. The initial body weights for humans were used in all calculations. The age at onset (measured in days), which was either reported or needed to be estimated from the body weight of the animal species, was added to the duration of exposure (also in days) to calculate the age at the midpoint of the experiment. A predictive regression model developed by Poiley's (1972) which is based on the animal species, strain, weight and age, was used to estimate the body weight for experimental animals of different ages.

If the study did not report specific information on the actual amount of copper consumed in the feed or water provided to the experimental subjects, average daily consumption of feed and water had to be estimated. The average daily intake of feed for rats was estimated from data provided by the National Academy of Sciences (NAS) (1972) and average daily water consumption was derived from the Canadian Council on Animal Care (CCAC) (1984). Average daily feed and water consumption for mice was derived from the Louisiana Veterinary Medical Association (LVMA) (2008). Table 3.3 lists the sources used

to obtain information on background levels of copper in diet, average daily feed and water consumption and average body weight.

Table 3.3 References Used to Estimate Copper Intake and Body Weight

Background level of copper in diet: Humans Baker, A., Harvey, L., Majask-Newman, G., Fairweather-Tait, S., Flynn, A., Cashman, K. 1999a. Effect of dietary copper intakes on biochemical markers of bone metabolism in healthy adult males. *Eur. J. Clin Nutr.* 53:408-412.
Average daily feed: Rats National Academy of Sciences. 1972. Nutrient requirements of laboratory animals. No. 10 in the series, "Nutrient Requirements of Domestic Animals." Washington, DC: National Academy of Sciences.
Body weight & age: Rats, Mice Poiley SM, 1972. Growth Tables for 66 Strains and Stocks of Laboratory Animals. *Lab. Ani. Sci.* 22:759.
Water consumption: Rats Canadian Council on Animal Care. 1984. Guide to the Care and Use of Experimental Animals. Volume 2. available: http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/Guidelis.htm.
Age: Rats Canadian Council on Animal Care. 1984. Guide to the Care and Use of Experimental Animals. Volume 2. available: http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/Guidelis.htm.
Feed & water consumption: Mice Louisiana Veterinary Medical Association. 2008. Biology of the Mouse. available: www.lvma.org/mouse.html
Feed consumption: Rabbits National Laboratory Animal Centre. Rabbit. 2001. Mahidol University. available: http://www.nlac.mahidol.ac.th/nlacmuEN/p_animal_Rabbit.htm

3.3 ENDPOINT ANALYSIS

In order to give a sense of the diverse number of responses reported in the studies available on copper excess and deficiency, responses associated with each dose group within each study were described. The distribution of experiments across the tissues and organ systems in which responses have been measured, and the types of responses most often associated with different levels of severity were summarized. Studies that measured longevity (mortality studies) were reviewed to ensure that a sufficient range of alternative

measures of toxicity were considered. In mortality studies, the investigators are interested in whether certain levels of a substance are associated with survival and may not measure other less severe indicators of copper toxicity. As survival may not be affected in all dose groups, if mortality is the only endpoint measured, a very high or low exposure to copper would be assigned a severity level 0. Had more sensitive measures been used, these levels of exposure would likely be associated with higher levels of severity. As a large number of mortality studies can underestimate the risk of copper toxicity, their impact on the exposure-response curve was assessed with sensitivity analyses if they did not report on other responses to elevated or deficient copper intake.

3.4 ACUTE EXPOSURE STUDIES

In the categorical regression analysis, the model fit was assessed with and without the inclusion of acute exposure studies. The pathways by which copper toxicity produces adverse effects in acute exposure studies are not the same as the pathways by which chronic or even subacute or subchronic exposures result in adverse effects (Stern et al., 2007). The range of endpoints measured and the study design can be quite different between these two types of studies.

3.5 DATA DESCRIPTION

Preliminary descriptive analyses of the copper toxicity database were conducted to characterize the extent of variability and scope in terms of the study design, range of exposures, and the distribution of observations by the animal species, age, route of exposure and sex. Descriptive analyses of the database helped determine whether there were a sufficient number of observations across the variables of interest to allow for the exposure-response model to be stratified by factors such as animal species, age, sex, or study design. The durations of exposure for rats, mice, and human were categorized as acute, subacute,

subchronic and chronic. Frequency counts of the total number of experiments in each category were computed for each animal species.

Frequency counts of the total number of experiments using drinking water versus feed were computed for each animal species for copper excess. It is important to note that copper ions are generally more bioavailable in water than in food; therefore, gastrointestinal (GI) irritation is more likely to be produced following uptake of copper in drinking water than food, as acute irritation of the GI tract is caused by the ionic form of copper. The majority of reports on GI irritation from copper are related to ingestion of fluids containing copper (NAS, 2000). Furthermore, if the same amount of copper is administered in feed in one experiment and in drinking water in another; more copper will be absorbed in the latter experiment. To account for the increased risk of toxicity from copper in drinking water, the exposure-response model may need to be stratified by the route of exposure (drinking water versus feed). Copper is often administered in drinking water in copper deficiency studies, in which the copper deficient groups are given purified drinking water and a diet without copper or, at the most, trace amounts of copper. The control group is then given the same diet; however, their drinking water is supplemented with adequate levels of copper to prevent any responses associated with copper excess or deficiency. Therefore, whether copper is administered in the drinking water or the diet of the control group will not impact the severity of response in copper deficiency studies.

Descriptive analyses were also conducted to describe the frequency of observations by sex. To give a sense of the distribution of observations by severity scores, the number observations by severity score and animal species were tabulated for data on both copper excess and deficiency.

Observations that correspond to experiments that have used less soluble forms of copper were identified in the database summary in order to evaluate whether there was a sufficient number of studies using less soluble forms of copper to create a two level variable for solubility ('low solubility' versus 'high solubility'). If there were insufficient numbers of studies using less soluble forms of copper, sensitivity analyses were used to evaluate the effect of solubility.

The distribution of observations in the database across different age groups was also tabulated in the database summary. At this time, the human studies in the database only focus on adults ($\geq$ 18 years of age). In order to stratify the intercept, concentration and/or duration parameters by age a categorical variable was defined. Age was categorized in two categories: 'young' and 'mature'. Young rats and mice were less than or equal to 30 days of age and mature rats and mice were greater than 30 days of age. These categories were based on age categories and life stage estimates from the Canadian Council on Animal Care (1984), which in turn is based on the estimated age at puberty.

3.6 CATREG SOFTWARE

CatReg, a software program developed by the U.S. Environmental Protection Agency, was used to conduct the exposure-response analysis. For the purposes of describing the technical background of exposure-response models in CatReg, Y will denote a dependent variable that represents the severity or intensity of the response. Y is assumed as being an ordinal score taking one of the values 0, 1 ...S. The lowest severity score would correspond with a value of 0 and a score of S would correspond with the highest severity score (6, in the present application). "Categorical regression is a method for modeling the probability distribution of Y as a function of the explanatory variables, concentration (C) and duration (T) (US EPA, 2000)." It uses a generalized linear model (McCullagh and Nelder, 1989) to

describe the dependence of the probabilities of different severity categories on the explanatory variables.

Two models are available in the program including a cumulative odds model (Equation 1) where the probability that severity level "s" or higher will occur at a given exposure concentration (C) and duration (T) is described by the categorical regression model. While the intercept parameters may differ by the severity level, the coefficients for C and T do not (US EPA, 2000). This model has the mathematical form:

$$\Pr(Y \geq s|C,T) = H[\alpha_s + \beta_1 * f_1(C) + \beta_2 * f_2(T)] \qquad \text{Equation 1}$$

The left side of the equation reads: "the probability that a response of severity level s or greater occurs, given that concentration is C and time is T (US EPA, 2000)." The right-hand side can be described as follows: "H is a cumulative probability function for which the user has three choices: (1) logistic, (2) normal, and (3) Gumbel. Current choices for *f* are "untransformed" or "base=10 logarithm". Model parameters include the intercept or severity parameter (α_s), the coefficient of concentration (β_1), and the coefficient of duration (β_2) (US EPA, 2000). The probability function, H, ensures that probability estimates are always between 0 and 1. In terms of severity (s) there is no expression for s=0 because this severity level represents the 'minimal category', and Y will always be greater than or equal to 0. The severity parameters must be correctly ordered ($\alpha_1 \geq \alpha_2 \geq \ldots \geq \alpha_s$). In CatReg this constraint is a consequence of the requirement that the probability of exceeding a lower score is larger than the probability of exceeding a higher score for any fixed values of C and T.

Unlike the cumulative odds model (Equation 1), the unrestricted cumulative model estimates separate coefficients for C and T at each severity level (Equation 2) (US EPA, 2000):

$$\Pr(Y \geq s|C,T) = H[\alpha_s + \beta_{1s} * f_1(C) + \beta_{2s} * f_2(T)] \qquad \text{Equation 2}$$

There are three choices for the probability function in the model: the normal and logistic distribution, which are symmetric, and the Gumbel distribution which is skewed. For each of the three choices of the probability function, H, there is an inverse function of H, called the link function, which transforms it to a simple linear function in concentration and duration. The use of the link function is important since without it, the linear model will become unbounded and one is led to meaningless estimates of probabilities for extreme values of C and T (US EPA, 2000). A more technical description of link functions as used in categorical regression is provided in Appendix B.

One of the important features in CatReg is the ability to stratify the regression parameters, which allows different subsets of the data to have different values for some or all of the parameters. In Appendix C, an example drawn from the CatReg documentation manual is presented to show how CatReg incorporates stratified variables into the exposure-response model (US EPA, 2000).

Another important feature of CatReg is the ability to calculate the extra risk concentration (ERCq) from the exposure-response model. The extra risk (ER) at concentration C=c and time T=t, at severity level s is defined by:

$$ERC = \Pr(Y \geq s \mid C=c, T=t) - \Pr(Y \geq s \mid C=0, T=t) \;/\; 1 - \Pr(Y \geq s \mid C=0, T=t) \qquad \text{Equation 3}$$

"For q (extra risk) between 1 and 100, inclusive, ERCq at time T=t, is the concentration c for which Equation 3 equals q/100 (US EPA, 2000)." For example, ERC10 at T=50 (exposure duration is 50 days) for severity level 2 is the value of c that satisfies Equation 4.

$$\frac{\Pr(Y \geq 2 \mid C=c, T=50) - \Pr(Y \geq 2 \mid C=0, T=50)}{1 - \Pr(Y \geq 2 \mid C=0, T=50)} = 0.1 \qquad \text{Equation 4}$$

In this example, the ERC10 at T = 50 for severity level 2 is the exposure concentration at which the probability is 0.10 of an adverse effect of level s or higher occurring following an exposure of 50 days, given that the adverse effect would not have occurred from other causes during this time (US EPA, 2000). CatReg can adjust for the fact that there may be a positive probability of an adverse effect even when concentration is zero (i.e., background response not attributed to the exposure). The user also has the option to assume that there is zero background risk.

It is important to note that if the data are based on individual exposed subjects, then probability represents actual risk. If the data is only available at the dose group level, which is the case in this analysis, then the ERCq represents the probability that one would assign the dose group as a whole to a severity level of s or greater (Hertzberg and Dourson, 1993).

One of the utilities of CatReg is the ability to use multiple independent variables to explain the response. There is often interest in how predicted effects change with the exposure duration. Haber's law (CxT) or a modification of Haber's law (C^nT) is one way to predict how effects change with the duration of exposure. These approaches are based on the assumption that the effect is constant if the product of concentration and time is a constant (i.e., C x T = constant), or if C^n x T= constant (Haber et al., 2001). After the exposure-response curve has been defined in CatReg, the C^nT can be defined. The value for n in C^nT can be estimated by dividing the coefficient for concentration by the coefficient for duration (β_1 / β_2). The program also allows us to test whether we can simplify our model ((α + $\beta_1$log(CT)) by testing whether the coefficient for concentration is equal to the coefficient for duration.

There are certain minimal data requirements that need to be satisfied in order to estimate the categorical regression model. One of the requirements is that there needs to be

at least one observation in each severity category. Furthermore, there are technical limitations on the complexity of the model that can be fit relative to the richness of the available data, including the fact that the model cannot include more parameters than the number of independent observations (US EPA, 2000).

The copper excess and deficiency data was modeled first with the cumulative odds model (Equation 1) followed by the unrestricted cumulative model (Equation 2). The unrestricted cumulative model is more complex as there are separate parameter estimates for concentration and duration for each level of severity. In CatReg the 'parallel.test' function was used to test the joint hypothesis that the parameter estimates for concentration for each severity level and the parameter estimates for duration for each severity level are equal. The test is a generalized Wald-type chi-square test of the null hypothesis that all of the specified constraints hold. A p-value less that 0.05 was considered as evidence that the hypothesis should be rejected and that the more complex model (i.e., unrestricted cumulative model) should be used. Assuming that the coefficients for concentration and duration are the same across all severity scores is a strict assumption. For example, the cumulative odds model (Equation 1) is assuming that the rate at which concentration and duration of exposure impact the risk of severity level 1 or greater (responses associated with homeostatic adaptations) is equal to the rate at which concentration and duration of exposure impact the risk of severity level 6 (death). As the unrestricted cumulative model (Equation 2) allows the effects of concentration and duration to vary by the severity level, separate coefficients for concentration and duration may need to be computed; however, this greatly increases the number of parameters in the model. As noted earlier, in both models there is an order constraint on the estimates for each severity level in both models. If the parameter

estimates for the severity levels are out of order (i.e., non-monotonic), this indicates that two or more severity levels might need to be combined (US EPA, 2000).

3.7 WEIGHTING THE DATA

A weighing factor based on group size was assigned to each dose group in the copper exposure-response database. As there are several responses of interest within each study and the majority of the response data is measured on a continuous scale, the copper toxicity database was built by assigning severity scores to group level data. With group level data, one severity score is assigned to the entire exposure group rather than to those subjects experiencing the event. In this context, incidence scoring refers to severity scores being assigned to the proportions of subjects experiencing and not experiencing the adverse effect. Severity scores were assigned to all responses measured for each exposure level in the experiment, with the highest severity score then selected to represent that level of exposure in the categorical regression analysis. With this approach we are assuming that the mean response is representative of the sample of subjects in the exposure group, irrespective of whether they experienced the event. Haber and associates (2001) comment on the fact that if the database contains incidence data only, then the ERCq is equivalent to risk.

The model used in the present analysis assumes highly correlated responses in individuals within a group and treats groups as individuals with an effective sample size of n=1 (Guth et al., 1997). However, when treating groups as individuals, the analysis ignores the possibility that some groups may be significantly larger than others. One study by Hertzberg et al. (1993) recommends the use of weighing factors assigned to different group sizes. Group size can be viewed as a quality indicator and assigned to one of a small number of weighting factors (Hertzberg et al., 1993). Table 3.4 presents the group size and

corresponding weighing factors proposed by Hertzberg et al., (1993). Each observation in the copper toxicity database was assigned a weighing factor as outlined in Table 3.4.

Table 3.4: Group Size and Weighing Factors

Group Size	*Weighing Factor*
1-10	1
11-25	2
26->	3

3.8 CLUSTER SAMPLES

In the copper toxicity database, there will be groups of observations from the same experiment and/or the same study. Ignoring these clusters of observations will likely lead to unduly narrow confidence bounds on the model parameters. CatReg provides an option for the user to specify whether or not the dataset contains any clusters. If clusters of data are given unique identifiers, CatReg assumes that responses from the same cluster are correlated, whereas observations from different clusters are independent. CatReg uses the method of generalized estimating equations to account for the cluster sampling effect (Simpson et al., 1996; Diggle, et al., 1994). Further information on generalized estimating equations from the CatReg user manual is provided in Appendix D. In this analysis, all observations from the same reference and experiment were treated as a cluster.

3.9 DOSE METRIC SELECTION

There is evidence that dose is more comparable between species when it is based on body surface area rather than body weight (Rhomberg and Lewandowski, 2006). The use of surface area assumes that the direct increase in body mass alone cannot account for interspecies differences in sensitivity (Calabrese et al., 1992). Travis and White (1988) provide empirical evidence to suggest that $bw^{3/4}$ compared to $bw^{2/3}$ provides a better estimate of actual surface area. It is important to note that the overall goal of selecting a common inter-species dose scaling metric is not necessarily to bring the estimates for rats,

mice and humans into line with one another. CatReg allows model parameters to be stratified, so that different subsets of the data can have different values for some or all of the parameters.

Hertzberg and Miller (1985) state that "if the data show substantially less species variation when using a transformed dose, then a prediction of human toxic levels from the animal studies should be improved." Hertzberg also comments on the fact that, "a common modeling problem is that if there is no constraint to the mathematical structure of the model, then small data sets can lead to extremely poor predictions, even when the curve fit is good" (Hertzberg and Miller, 1985). Therefore there is a need to constrain the model structure to some class of biologically plausible functions.

Five metrics were compared in this analysis including mg/day, mg/kg bw/day, mg/kg $bw^{1/4}$/day, mg/kg $bw^{2/3}$/day and mg/kg $bw^{3/4}$/day. The metric associated with the lowest AIC was selected for further analyses.

3.10 STRATIFICATION AND TRANSFORMATION OPTIONS

Choosing the distribution function and deciding whether to transform concentration and duration was treated as an empirical issue. To select the link function and the transformation options for concentration and duration, the AIC was used to compare 12 different models defined by three different link functions (logit, probit and complementry log-log) and four transformation options (log or linear selection of concentration and/or duration parameters). The selection of the link function is a modeling decision that currently does not have a biological basis. The model with the lowest value for the AIC was thus selected for further analyses.

The effects of potentially important explanatory variables can be assessed in CatReg by stratifying parameters according to the levels of these variables. Stratification allows

systematically different subsets of the data to have different values for some or all of the parameters (US EPA, 2000). Upon stratifying the model's intercept, concentration and/or duration parameters CatReg provides an option to test whether the estimates produced for one variable (e.g., intercent coefficients for rats, mice and humans) are statistically different from each other. The hypothesis to be tested is expressed as a set of constraints on the model coefficients. For example, we might be interested in whether the estimates for the concentration parameter stratified by the animal species (i.e. humans (HU), rats (RT) and mice (MU)) are statistically different. The test that is conducted is a generalized Wald-type chi-square statistic of the null hypothesis that all of the specified constraints hold. The distribution of the test statistic is derived from the sampling distribution of the estimated model coefficients, taking into account any cluster sampling effects. A p-value of less than 0.05 was taken as evidence that the null hypothesis can be rejected. The same strategy was used to test whether any neighbouring intercept parameters (defined for each level of severity) should be combined.

Model selection was based on a series of likelihood ratio tests between nested models. The goal was to produce an exposure-response curve that is sufficiently accurate by considering different parameters and stratification options, but one that achieves this aim as simply as possible. If the stratification of any of the parameters decreased the AIC by only a small amount, the simpler model was used. A likelihood ratio test for the significance of the more general model was computed by taking the difference between the deviances of the two models. The difference in the deviance was compared to a chi-square distribution with degrees of freedom equal to the differences in the number of parameters between the two models.

The effect of the animal species, route of exposure (drinking water versus feed), age, and sex on the model parameters was investigated. As mentioned in section 3.5, whether copper is administered in the drinking water or the diet of the control group will not impact the severity of response of the experimental group in copper deficiency studies. Therefore, the possible effect of route of exposure was only examined in the analysis of the copper excess data.

There is an issue in defining the variable for sex as there are studies that do not report results independently for males and females. There are two approaches to addressing this issue. One can create three categories ('both', 'males' and 'females') and then examine the effect of sex in the model. The effect of a three-level sex variable in the exposure-response model can be verified by temporarily filtering out the observations that do not report the sex of the animal species in order to create a variable with only two categories ('males' versus 'females'). Both strategies were compared to look at the effect of sex in the model.

3.11 SENSITIVITY ANALYSES

Sensitivity analyses were conducted to look at how the model fit or the final estimates change when certain observations were excluded from the analysis. There is considerable variability in the copper toxicity database in terms of the animal species, strain, study design, age, sex, and target organ of observed toxicity that could not be fully accounted for by stratifying the exposure-response model as there may be insufficient numbers of observations across the levels of these variables. Studies that were identified during the endpoint analysis of the copper exposure-response database as having a limited range of endpoints (e.g., mortality studies) or unique study designs were selectively filtered from the analysis to investigate the effects on the ERCq and the parameter estimates.

3.12 EVALUATING MODEL FIT AND ASSUMPTIONS

One important consideration is the selection of the cumulative odds model versus the unrestricted cumulative model. The unrestricted cumulative model (Equation 2) is more complex and allows the severity parameters to vary by concentration and duration. This modeling option requires more data to converge on a solution as more parameters are estimated. The cumulative odds model (Equation 1) is less complex and does not allow the severity parameters to vary by concentration and duration which has the effect of constraining the model predictions for different severity categories to be parallel (Guth, 1997). The unrestricted cumulative modeling option was used to test whether the simpler model (the cumulative odds model) was adequate. CatReg allows one to test whether the model parameters for concentration and duration at each severity level in the unrestricted cumulative model are equal. A p-value of less than 0.05 was considered as evidence that the data does not conform to the assumption of parallelism and thus the more complex model should be used. The test is a generalized Wald-type chi-square test of the null hypothesis that all of the specified constraints hold. The distribution of this test statistic is derived from the sampling distribution of the estimated model coefficients, and takes into account cluster sampling.

There are several options in CatReg for evaluating model fit and identifying observations that appear to be outliers. CatReg uses the individual components of the deviance statistic to measure how well individual observations are explained by the exposure-response curve. Observations that contribute to any lack of fit of the exposure-response curve can be identified by examining the individual contributions to the deviance. Any data points identified as potential outliners were reviewed in terms of the corresponding study design and range of endpoints measured. There appears to be a program error in CatReg

around the use of the deviance plotting function. Only data points for up to five strata appear in the plots generated in the program. If there are for example eight strata defined by animal species, route of copper exposure and age, observations from the last three strata will not be plotted. As the strata are read in alphabetical order, using the example above where the model was stratified by animal species, route of copper exposure, and age, the data from the three strata on rats would not appear in the deviance plot. In order to observe the data points for all the strata, two figures were created. To generate the second plot, the data file was modified so that the three rat strata are read in first. In the end, between the two figures all the observations in the dataset were plotted by their observation number and their residual deviance.

CatReg was used to generate what the program refers to as 'catplots', which define the ERCq curves for a specified extra risk (q) at a defined level of severity by concentration and duration of exposure. These plots were used to identify any overlap in observed toxicity at a defined concentration and duration of exposure. They were also used to look at the impact of duration. CatReg also has a modeling function referred to as 'allsevsplots'. This modeling function plots the ERCq lines for each level of severity at a defined extra risk level on one graph where the y axis is defined by concentration and the x axis by duration. These figures can help identify which ERCq curves have been defined by extrapolating information from other strata. When there are no observations that correspond to a particular severity score, the ERCq line must have been defined by using observations from other strata in the analysis. These plots were also used to look at the difference in the magnitude of the ERCq lines between different severity levels. They were also used to qualitatively assess model fit. If the model adequately described the exposure-response data on copper excess, all ERC10 lines should be below their corresponding observations. For example the ERC10 for

severity level 2 or greater should be below all observations that have been assigned a severity level 2. If the model adequately describes the exposure-response data on copper deficiency, all ERCq lines should be above their corresponding observations.

The CatReg user manual comments on the fact that cluster sampling invalidates the large sample F distribution of the generalized F-statistic; however, it is common practice to compute F as a rough guide (Venables et. al., 1994; US EPA, 2000). As the coefficient of determination (R^2) can give a sense of the explanatory capacity of the exposure-response model, it was used as a rough guide to compare the fit of the unrestricted cumulative model and the cumulative odds model of the copper excess and deficiency data.

3.13 FINAL ESTIMATES

The previous sections have described the steps that were involved in defining an exposure-response model that provided the most appropriate description of the data in the copper exposure-response database. The steps thus far have allowed us to: evaluate the impact of incorporating acute exposure studies in a common analysis with subacute, subchronic and chronic exposure studies; evaluate the impact of the animal species, age, sex, and route of exposure on the risk of adverse effects from copper excess and deficiency; and characterize the exposure-response relationship for copper excess and deficiency. Once a model was defined with appropriate transformation, link functions and stratification options, CatReg was used to generate estimates (see Equation 4) for the ERC10 at severity level 2 or greater for the human stratum with the copper excess data as well as the copper deficiency data. This allowed us to define the limits of the acceptable range of oral intake at different risk probabilities and determine the magnitude of the difference between the ERC10 estimates between animal species. Estimates were presented for different levels of severity for this stratum and across different durations of exposure. For each stratum, ERC10

estimates for severity level 2 or greater were compared. In order to estimate an ERC at a defined level of probability, the duration must be fixed. The same duration was selected for copper excess and deficiency. A chronic duration of exposure would have been ideal; however, there is very limited data on humans after 100 days of exposure. Haber and associates (2001) commented on the fact that extrapolating beyond the data increases the model dependence as models that appear to fit equally well in the range of the data can have widely different results at doses several magnitudes lower or higher (Haber et al., 2001). The selection of the duration of exposure was based on the availability of data at subchronic and chronic durations of exposure and whether duration had an effect in the exposure-response model. Final ERC10 estimates at severity level 2 or greater (i.e., approximate of the acceptable range of oral intake) were compared with current recommendations for copper including the tolerable upper intake level and the RDI.

PART 4: RESULTS

4.1 EXPOSURES AND ASSUMPTIONS

Tables E1 and E2 in Appendix E present the estimates and assumptions used to define the body weight, feed intake, and water intake for each experiment that is included in the exposure-response database on copper excess and deficiency, respectively. Bolded values represent those values that had to be estimated. Values that are not bolded were directly recorded from the study. For those studies in which values had to be estimated, a brief description of the estimation process is provided. Information in Table E1 and E2 was used to calculate body weight at the midpoint of the experiment and the daily dose in milligrams per day. Tables E3 and E4 present the final estimates defined in mg/day, mg/kg bw/day, mg/kg $bw^{1/4}$/day, mg/kg $bw^{2/3}$/day, and mg/kg $bw^{3/4}$/day.

4.2 DATABASE SUMMARY

There are currently 79 studies and 210 observations on copper deficiency and 41 studies and 241 observations on copper excess in the copper database. An observation corresponds to each dose level from each study that was assigned a severity score. The majority of observations in the database come from studies on rats, followed by mice, and then humans. There are also a few studies on pigs and rabbits. Table 4.1 presents the number of observations for each animal species separately for the copper excess dietary studies, copper excess drinking water studies, and copper deficiency studies.

Table 4.1: Number of Observations by Animal Species

Study Category	# of Observations				
	Rats	*Mice*	*Humans*	*Pig*	*Rabbits*
Copper Excess Dietary Studies	105	26	21	20	2
Copper Excess Drinking Water Studies	25	19	25	0	0
Copper Deficiency Studies	173	22	13	2	0

After reviewing the range of durations of exposure used in each study, four exposure categories were defined, representing acute, subacute, subchronic, and chronic exposures. There are a small number of acute exposure studies on humans. In these studies, the investigators used a single daily dose of copper in drinking water and were primarily interested in the effects of elevated copper intake on gastro-intestinal symptoms (Araya et al., 2001; 2003a; 2003b; Gotteland et al., 2001; Jantsch et al., 1985). Subacute exposures ranged from 7 days to 2 months in duration, and subchronic exposures ranged from 2.5 to 4 months. Chronic exposures were defined as greater than 6 months in duration.

Table 4.2 presents the number of observations by the animal species and the exposure category for the copper excess dietary studies, copper excess drinking water studies, and the copper deficiency studies.

Table 4.2: Number of Observations by Animal Species and Duration of Exposure

Study Category	Exposure Category	# of Observations				
		Humans	*Rats*	*Mice*	*Pigs*	*Rabbits*
Copper Excess:						
Dietary	Acute, <24h	1	0	0	0	0
	Subacute, 7 days - 2 months	17	64	14	20	0
	Subchronic, 2.5 - 4 months	2	37	12	0	2
	Chronic, 6 months or greater	1	4	0	0	0
Drinking Water	Acute, <24h	13	0	0	--	--
	Subacute, 7 days - 2 months	8	23	12	--	--
	Subchronic, 2.5 - 4 months	4	2	5	--	--
	Chronic, 6 months or greater	0	0	2	--	--
Copper Deficiency:	Acute, <24h	0	0	0	0	--
	Subacute, 7 days - 2 months	5	48	2	0	--
	Subchronic, 2.5 - 4 months	5	108	15	2	--
	Chronic, 6 months or greater	3	17	5	0	--

The majority of human studies on copper excess utilize copper sulfate. There are two studies in the database that use copper glycine chelates (Jones et al., 1997; O'Connor et al., 2003). Chelated minerals are often used to increase the efficiency of absorption and glycine is an amino acid that the body readily identifies and is absorbed efficiently across the intestinal wall. In O'Connor's study there were two experiments, one using copper glycine chelates and the other using copper sulfate (2003). There were no differences between these two experiments in the severity of adverse effects. In copper deficiency studies, the diets of the experimental groups contain very low amounts of copper. In human studies copper deficient diets often provide only 0.7 mg Cu/day. The control group often consumes the same diet but their drinking water is supplemented with copper. The majority of copper deficiency studies on humans have used copper sulfate; however, one study used copper chloride. Copper chloride, copper sulfate, copper gluconate and copper acetate are all examples of salts that are highly soluble.

The rat studies on copper excess also tended to use more soluble forms of copper including copper sulfate. One rat study on copper excess used copper acetate (Gross, 1989).

In Liu's study (1986) copper carbonate was used. Copper carbonate is a form of copper that is known to be less soluble compared to copper sulfate (Liu 1986). A sensitivity analysis will be conducted in section 4.5B and section 4.6D to look at the impact of this study on the extra risk concentration (ERCq) (Equation 4) for rats. One would expect that if there were two experiments using equal exposure levels where one used copper sulfate and the other used copper carbonate, the latter experiment would result in a lower severity level as less copper would be absorbed in the gastrointestinal tract.

The majority of rat studies on copper deficiency use copper sulfate. Two studies in the database use copper chloride (Sugawara 1999; Harvey 2003). Copper carbonate, the less soluble form of copper has been used in several rat studies on copper deficiency (Allen 1996; Bala 1990; Davidson 1992; Gomi 1995; Hopkins 1995; Mao 1999; Rock 1995; Wang 1996; Rayssiguier 1993; Ajayi 2005; Klaahsen 2007). The majority of the studies in the copper database on mice use copper sulfate; however, two mice studies use copper carbonate (Auclair 2006; Merino 1986).

Overall there appears to be several studies among the copper deficiency data using less soluble forms of copper. The exposure-response model could be stratified by a two-level solubility variable (low solubility versus high solubility); however, there are several studies that have not reported the form of copper used. As the majority of studies have used more soluble forms of copper such as copper sulfate, these observations will be categorized in the 'high solubility' group.

Table 4.3 presents the frequency of observations for two age categories for rats and mice. All studies in the database on humans focus on adults ($\geq$ 18 years of age). For experiments using rats, the majority of studies on copper excess and deficiency are on young rats ($\leq$30 days of age). The majority of studies on copper excess have used young mice;

however, for copper deficiency there are more studies on mature mice (>30 days of age). All studies on pigs have used young animals (≤30 days of age).

Table 4.3: Copper Excess and Deficiency Studies on Rats and Mice - Number of Observations by Age Category

Animal Specie	*Age Group**	# of Observations	
		Copper Excess	*Copper Deficiency*
Rats	Young ≤30 days	65	142
	Mature >30 days	65	31
Mice	Young ≤30 days	2	5
	Mature >30 days	43	17

*Age group classification refers to the age of the animal subjects at the onset of the experiment

Table 4.4 presents the frequency of observations by sex and animal species for the copper excess and deficiency data.

Table 4.4: Number of Observations by Animal Species and Sex

Study Category	*Animal Species*	# of Observations		
		Male	*Female*	*Both**
Copper Excess	Rats	95	27	8
	Mice	27	18	0
	Humans	10	12	24
	Pigs	0	0	20
	Rabbits	2	0	0
Copper Deficiency	Rats	139	20	14
	Mice	7	13	2
	Humans	13	0	0
	Pigs	0	0	2

* Study did not report results separately for males and females

All of the studies on rats, mice, pigs, and rabbits in the database have a common study design involving a series of independent exposure groups. For humans, 16 of the 23 studies in the database have a repeated cross-over design; five studies use independent exposure groups and two studies are case reports.

Table 4.5 presents the frequency of observations by animal species and severity score. Overall, there are very few observations that correspond to severity scores 5 and 6. There are also very few observations that correspond with severity score 1 for the copper excess data. The majority of observations fall in severity categories 0, 3 and 4 for copper excess and

0, 2 and 3 for copper deficiency. For copper deficiency, severity scores 5 and 6 will need to be combined in the analysis as there are no observations that correspond with a severity level 5.

Table 4.5: Number of Observations by Severity Level (SEV) and Animal Species

Study Category	Species	# of Observations						
		SEV 0	*SEV 1*	*SEV 2*	*SEV 3*	*SEV 4*	*SEV 5*	*SEV 6*
Copper Excess:								
Diet	Humans	14	0	5	0	2	0	0
	Rats	40	6	3	9	44	3	0
	Mice	13	0	0	1	12	0	0
	Pigs	12	0	3	5	0	0	0
	Rabbits	1	0	0	1	0	0	0
Drinking Water	Humans	14	0	0	0	11	0	0
	Rats	13	0	0	5	3	0	4
	Mice	8	0	0	4	2	0	5
Copper Deficiency:								
	Humans	5	3	3	2	0	0	0
	Rats	73	10	20	63	7	0	1
	Mice	11	0	1	6	4	0	0
	Pigs	1	0	0	1	0	0	0

Figures 4.1a-i present all the copper excess and deficiency data, the copper excess data alone, and the copper deficiency data alone for humans (a-c), rats (d-f) and mice (g-i), respectively. The copper excess data has a wider range of exposure durations compared to the data on copper deficiency. Studies on rats and mice have used a wider range of doses and durations of exposure than human studies. These figures emphasize the fact that the majority of the data will be coming from rat studies. Data that is clustered at T=1 corresponds with acute exposure studies.

Figures 4.2a-c present all of the observations on copper excess and deficiency defined by concentration (mg/kg bw/day) and duration (days) for rats, mice and humans, respectively. The observations are also defined by their level of severity. Each severity level is represented by the same symbol for both copper deficiency and copper excess. What should be seen is observations corresponding with severity level 0 clustering around the

middle of each figure and more observations corresponding with higher severity scores clustering around the extremes of copper excess and deficiency.

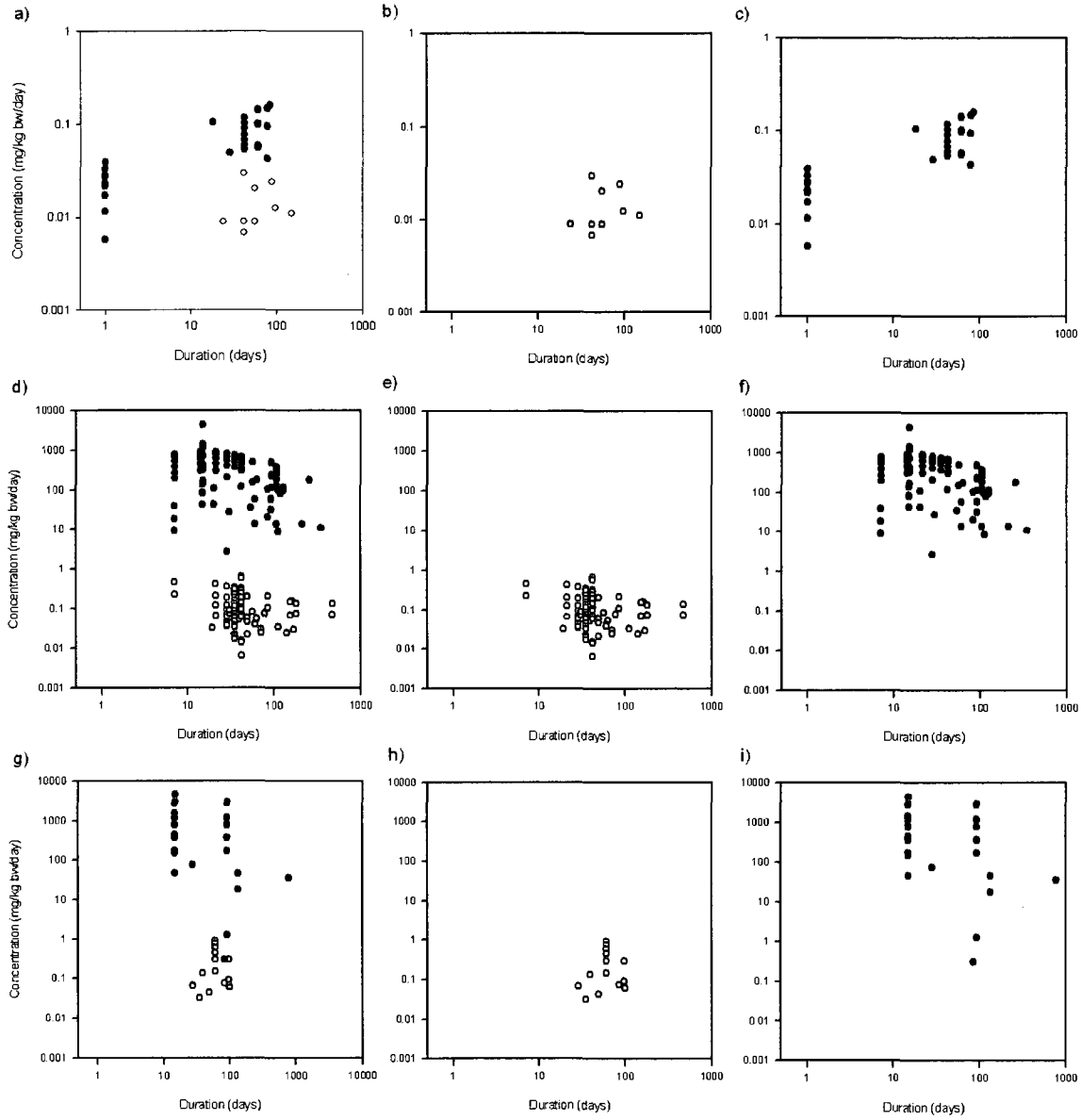


Figure 4.1a-i: Observations on Copper Excess and Deficiency by Concentration and Duration. Copper excess and deficiency data, copper deficiency data and copper excess data on humans (a-c), rats (d-f) and mice (g-i), respectively by concentration (mg/kg bw/day) and duration (days). Copper deficiency = ○, copper excess = ●.

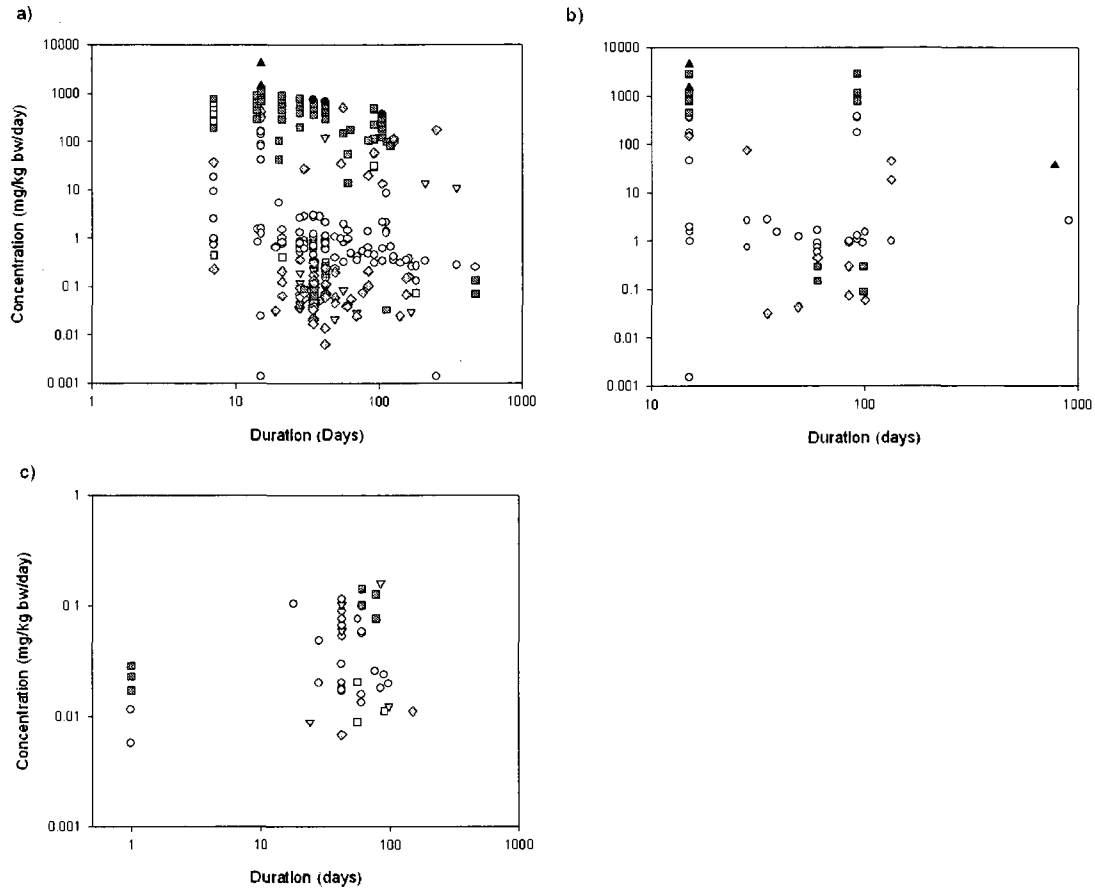


Figure 4.2a-c: Copper Excess and Deficiency Data by Concentration, Duration and Severity. Both copper excess and deficiency data, on rats (a), mice (b) and humans (c), by concentration (mg/kg bw/day), duration (days) and severity. Data points represented by○ = severity level 0,□ = severity level 1,▽ = severity level 2,◇ = severity level 3,▩ = severity level 4,● = severity level 5, and▼ = severity level 6

4.3 ENDPOINT ANALYSIS

4.3A Range of Responses Associated with Copper Excess and Deficiency

To give a sense of the broad range of responses associated with elevated or deficient levels of copper across experiments within the copper database, Tables F1 and F2 in Appendix F present information on each experiment, including the responses found to be associated with elevated or deficient levels of copper.

4.3B Organs Systems Associated With Copper Excess and Deficiency

Tables 4.6 and 4.7 list the frequency of experiments for different categories of responses. For copper excess (Table 4.6), the majority of experiments look at responses

associated with the liver, renal system, haematopoietic system, the gastrointestinal system, brain, respiratory system and the cardiovascular system. There are fewer experiments that have looked at responses associated with the nervous system, reproductive system, the thymus, bone, and hair.

For copper deficiency (Table 4.7), the majority of experiments have looked at responses associated with the blood, hepatic system, cardiovascular system, renal system, the gastro-intestinal system, and the spleen. Fewer experiments have looked at responses associated with the adrenal gland, bone, and pancreas. While body weight change and mortality are endpoints used in several studies on copper excess and deficiency they are not necessarily associated with one particular target organ. Body weight has been a response of interest in 22 experiments on copper excess and 45 experiments on copper deficiency. Mortality has been a response of interest in 4 experiments on copper excess and 2 experiments on copper deficiency.

Table 4.6: Frequency of Experiments by Target Organ – Copper Excess

Endpoint	*Frequency of Experiments*
Hepatic	44
Renal	31
Haematopoietic system	30
Gastrointestinal	17
Brain	8
Respiratory	6
Cardiovascular	6
Neurological	4
Skeletal	4
Hair	2
Reproductive	2
Thymus	1

Table 4.7: Frequency of Experiments by Target Organ – Copper Deficiency

Endpoint	*Frequency of Experiments*
Haematopoietic	73
Hepatic	69
Cardiovascular	49
Renal/Urinary	17
Gastrointestinal	6
Spleen	6
Muscle	4
Brain	4
Respiratory	4
Adrenal gland	1
Bone	1
Pancreas	1

4.3C Adverse Responses Associated with each Severity Level

For copper excess, the most severe severity score (severity level 6) corresponded with mortality. Severity level 5 has only been associated with chronic hepatitis. Severity level 4 was most often associated with histopathological changes in the liver and kidney, as well as gastro-intestinal hyperplasia, gastro-intestinal symptoms, brain function, and altered aortic morphology.

For copper deficiency, the most severe severity score (severity level 6) also corresponded with mortality. No observations were assigned a severity level 5. For severity level 4, responses were most often associated with toxic effects in the cardiovascular system and the reproductive system.

For both copper excess and deficiency, severity level 3 was most often associated with perturbed mineral metabolism, body weight changes, altered levels of hematocrit and hemoglobin, altered levels of immune cells, changes in enzyme activities, altered neurotransmitter levels in the brain and changes in organ weight. Severity level 2 was most often associated with altered levels of carrier proteins and enzyme levels. Severity level 1 was most often associated with altered levels of copper in different target organs.

4.3D Mortality Studies

As discussed in Part 3, a large number of mortality studies that do not measure other responses to elevated or deficient copper intake can underestimate the risk of copper toxicity. There are no studies in the database that examined only mortality as an endpoint. Among the copper excess studies, several of the experiments by Hebert (1993) have looked at mortality; however, these experiments have also looked at histopathological changes, clinical signs, body weight, and responses associated with the renal system. Massie and Aiello (1984) looked at mortality but also responses associated with the hematopoietic system, the renal system, body weight, the liver, the nervous system, and the cardiovascular system.

4.3E Drinking Water Studies

For the human data, observations from studies where copper was administered in drinking water only corresponded to severity level 0 and severity level 4. While these drinking water studies have measured markers of copper imbalance that could be influenced by subchronic or chronic exposures to excess levels of copper (i.e., hemoglobin, serum Cu burden, caeruloplasmin and serum enzymes), the only responses associated with a severity level 4 have been recurrent gastrointestinal symptoms (e.g., nausea). Gastrointestinal symptoms are likely only a result of acute ingestion of the copper solution rather than chronic effects of long-term copper intake. The impact of these studies on ERC10 estimates for humans will be investigated in the sensitivity analysis in section 4.5B and 4.6D.

4.4 MODEL SELECTION FOR COPPER EXCESS – METRIC, TRANSFORMATION AND LINK FUNCTION

This section selects the most appropriate link function and decides whether concentration or duration should be transformed. This section also demonstrates how using different dose metrics impacts the model deviance. The unstratified cumulative odds model (Equation 1) was used for these comparisons. Pig and rabbit studies were omitted from the

analysis due to their scarcity in the database. Acute exposure studies were also omitted from the analysis; however, section 4.5B evaluates the impact of including the acute exposure studies in the exposure-response model. The pathways by which copper toxicity produces adverse effects and the range of resulting endpoints are different in acute exposure studies. Their addition will likely reduce or eliminate any effect of duration in the exposure-response model.

In Table 4.8, the AIC is listed for the 12 different modeling options defined by three different link functions (logit, probit and C log-log); two transformation options (log and linear); and 5 different dose metrics (mg/day, mg/kg bw/day, mg/kg $bw^{1/4}$/day, mg/kg $bw^{2/3}$/day, and mg/kg $bw^{3/4}$/day).

Table 4.8: AIC for 12 Modeling Options and 5 Dose Metrics for Copper Excess

Link Function	*C*	*T*	AIC for Each Dose Metric				
			Mg/d	*Mg/kg bw/d*	*Mg/kg $bw^{1/4}$/d*	*Mg/kg $bw^{2/3}$/d*	*Mg/kg $bw^{3/4}$/d*
Logit	Linear	Linear	548.19	576.76	532.95	541.93	549.37
Logit	Linear	Log	548.01	574.78	531.42	538.80	546.25
Logit	Log	Linear	544.45	547.11	529.22	530.39	534.58
Logit	Log	Log	544.15	541.52	527.44	525.57	528.73
Probit	Linear	Linear	559.81	579.49	539.21	542.09	550.92
Probit	Linear	Log	562.19	579.79	541.33	541.60	550.55
Probit	Log	Linear	555.88	574.04	535.10	529.40	534.00
Probit	Log	Log	558.24	543.70	537.08	527.55	531.86
C Log-log	Linear	Linear	581.33	NA*	554.33	539.53	547.98
C Log-log	Linear	Log	582.51	NA*	555.60	538.35	547.40
C Log-log	Log	Linear	577.34	NA*	550.35	529.00	532.53
C Log-log	Log	Log	578.52	NA*	551.54	525.92	529.79

*Not suitable link function
Note. C, concentration; T, duration; C Log-Log, complementry log-log

When the dose is defined in mg/kg bw/day the selection of the complementry log-log link function (C log log) in CatReg produces several error messages. The CatReg user manual states that the complementry log-log link function tends to be easier to overparameterize than the other link functions. This means that it cannot handle as many variables because of the simplicity of the log-log transformation and the linear relationship that results from the use of this transformation. The program recommends the use of the

logit or probit link function when the use of the C log-log link function produces these errors messages. For every dose metric the lowest AIC was found to result from transforming concentration and duration (log10) and using the logit link function. The lowest AIC corresponded to the model where concentration was defined as mg/kg $bw^{2/3}$/day. All modeling options in section 4.5 and 4.6 will be based on the logarithm (log10) of concentration defined in mg/kg $bw^{2/3}$/day and the logarithm (log10) of duration defined in days. All models will use the logit link function.

4.5 CUMULATIVE ODDS MODEL – COPPER EXCESS

In the following subsection (4.5A) a series of stratification options were compared in order to select a model that is sufficiently accurate by considering different parameters (i.e. animal species, route of exposure, age and sex) and stratification options (i.e. stratification of the intercept, concentration and/or duration parameter).

4.5A Model Selection – Stratification Options

The copper excess data was first fit with the cumulative odds model. Table 4.9 presents the estimated parameters of the fitted exposure-response models, their standard errors, and significance level associated with the null hypothesis that the true value of the parameter is zero.

Table 4.9 Parameter Estimates, Standard Errors, Z-test Statistics and P-values for Copper Excess Dietary Studies Using the Cumulative Odds Model*

Parameter	*Estimate*	*Std. Error*	*Z-test*	*P-value*
SEV1	-2.7592	0.6875	-4.0132	<0.0001
SEV2	-3.1392	0.7162	-4.3832	<0.0001
SEV3	-3.3339	0.7057	-4.7243	<0.0001
SEV4	-4.0036	0.7674	-5.2171	<0.0001
SEV5	-7.2377	0.8243	-8.7806	<0.0001
SEV6	-7.5712	0.8597	-8.8064	<0.0001
LG10CONC	1.38862	0.2136	6.4907	<0.0001
LG10TIME	1.2035	0.3586	3.3566	0.0008

* Cumulative odds model uses the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) have been log transformed to the base 10. Note. SEV, severity level; LG10, log transformed to the base 10; CONC, concentration coefficient; TIME, duration coefficient.

Separate intercept coefficients are estimated for each level of severity. Table 4.10 presents the results from a generalized Wald-type chi-square test of the null hypothesis that the parameter estimates for neighboring severity scores are equal. The p-values for the first four combinations of severity scores are significant, providing evidence that these parameters are not equal. The p-value for the equality test of severity levels 5 and 6 is 0.0785 which is just above the nominal level of 0.05. While the p-value is not below 0.05, a value this low does provide some evidence of a difference between severity levels 5 and 6. The analysis will proceed using all six levels of severity.

Table 4.10: Equality Test for Neighboring Severity Coefficients – Copper Excess

Test of Equality	*Chi-square*	*df*	*P-value*
SEV1 = SEV2	7.5470	1	0.0063
SEV2 = SEV3	4.3328	1	0.0374
SEV3 = SEV4	11.4850	1	0.0007
SEV4 = SEV5	99.7925	1	<0.0001
SEV5 = SEV6	3.0949	1	0.0785

Cumulative odds model uses the logit link function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) transformed (log10). Note. SEV, severity level.

The AIC from a series of models were compared to look at the impact of the animal species on the intercept, concentration, and duration parameters (Table 4.11). The upper column lists the parameter in the model (intercept, concentration and duration parameter), the rows list the model number and the cells list what has been stratified in each model. For example, in model 2, the intercept is stratified by animal species, whereas in model 5, both the concentration and duration parameter are stratified by animal species.

Table 4.11: AIC Comparison of Eight Modeling Options Defined by the Stratification of the Intercept, Concentration and/or Duration Parameter by the Animal Species

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1	--	--	--	501.0888
2	Animal species	--	--	443.0426
3		Animal species		500.1378
4			Animal species	445.8157
5		Animal species	Animal species	444.7849
6	Animal species		Animal species	446.7253
7	Animal species	Animal species		438.1089
8	Animal species	Animal species	Animal species	441.3585

Cumulative odds model is used in all eight models with the logit link function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) transformed (log10).

Model 7, where the intercept and concentration parameters have been stratified by animal species, is associated with the lowest AIC; however, a Wald-type chi-square test of the null hypothesis that the parameters that have been stratified are equal is not significant for the concentration parameter (Table 4.12). In model 8, which is associated with the second lowest AIC, the effect of the animal species on the intercept and duration parameters is not significant (Table 4.12). Model 2, where the intercept is stratified by the animal species is associated with the third lowest AIC. A Wald-type chi-square test of the null hypothesis that the parameters that have been stratified are equal is significant ($p<0.0001$) (Table 4.12). To determine whether the difference in the AIC between model 2 and the unstratified model (model 1) is large enough to justify the use of a more complex model, the difference between the deviance of model 2 and model 1 (unstratified model) was tested against a chi-square distribution with 2 degrees of freedom ($df_{model2} = 4$, $df_{model1} = 2$). The difference between the deviances of the two models (487.0888 - 425.0426=64.0462) is significant ($p<0.0001$, $df = 2$).

Table 4.12: Test for the Effect of Animal Species in Models 7, 8 and 2

Model # and Stratification Options	*Chi-square*	*df*	*P-value*
Model 7:			
Intercept Stratified by Animal Species	16.7159	2	0.0002
Concentration Stratified by Animal Species	2.7631	2	0.2512
Model 8:			
Intercept Stratified by Animal Species	4.8292	2	0.0894
Concentration Stratified by Animal Species	5.9866	2	0.0501
Duration Stratified by Animal Species	0.3460	2	0.8411
Model 2:			
Intercept Stratified by Animal Species	18.5347	2	<0.0001

Cumulative odds model is used in all 3 models with the logit link function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) transformed (log10).

The AIC from a series of models were compared to look at the impact of the route of exposure (i.e., drinking water or dietary exposure) on the intercept, concentration and/or duration parameter (Table 4.13). In every model, the intercept is also stratified by the animal species.

Table 4.13: AIC Comparison of Eight Modeling Options Defined by the Stratification of the Intercept, Concentration and/or Duration Parameter by the Route of Exposure

Model #	Intercept	Concentration	Duration	AIC
1	Animal Species	--	--	443.0426
2	Animal Species Route of Exposure	--	--	411.5931
3	Animal Species	Route of Exposure	--	433.706
4	Animal Species	--	Route of Exposure	415.5907
5	Animal Species Route of Exposure	--	Route of Exposure	411.5340
6	Animal Species Route of Exposure	Route of Exposure	--	413.1435
7	Animal Species	Route of Exposure	Route of Exposure	415.9844
8	Animal Species Route of Exposure	Route of Exposure	Route of Exposure	409.6455

Cumulative odds model is used in all eight models with the logit link function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) transformed (log10).

Model 8, where the intercept, concentration and duration parameters are stratified by the route of exposure, is associated with the lowest AIC. A Wald-type chi-square test of the null hypothesis that the parameters that have been stratified are equal is not significant for all stratification options except for the effect of the animal species on the intercept (Table 4.14). While model 5 is associated with the second lowest AIC, the route of exposure does not have a significant effect on the duration or intercept parameter (Table 4.14). In Model 2 the effect of the route of exposure and the animal species on the intercept is significant (Table 4.14). The difference between the deviances of models 1 and 2 were tested against a chi-square distribution with 3 degrees of freedom ($df_{model2} = 7$, $df_{model1} = 4$). The difference between the deviances of the two models (443.0426-387.5931=55.4495) is significant ($p<0.0001$, $df = 3$). In further analyses the intercept will be stratified by the animal species and the route of exposure.

Table 4.14: Test for Effect of the Route of Exposure and Animal Species in Models 8, 5 and 2

Model # and Stratification Options	Chi-square	df	P-value
Model 8:			
Intercept Stratified by Route of Exposure[a]	5.4103	3	0.1441
Concentration Stratified by Route of Exposure	0.6526	1	0.4192
Duration Stratified by Route of Exposure	2.1800	1	0.1398
Intercept Stratified by Animal Species[b]	23.4856	4	0.0001
Model 5:			
Intercept Stratified by Route of Exposure[a]	7.4967	3	0.0576
Duration Stratified by Route of Exposure	2.1964	1	0.1383
Intercept Stratified by Animal Species[b]	19.4390	4	0.0006
Model 2:			
Intercept Stratified by Route of Exposure[a]	25.6092	3	<0.0001
Intercept Stratified by Animal Species[b]	21.4364	4	<0.0003

Cumulative odds model is used in all models with the logit link function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) are transformed (log10).
[a]Controlling for animal species
[b]Controlling for the route of exposure

Table 4.15 presents the AIC for eight models where the intercept, concentration and/or duration parameter was stratified by age. In every model, the intercept parameter is stratified by the animal species and the route of exposure.

Table 4.15: AIC Comparison of Eight Modeling Options Defined by the Stratification of the Intercept, Concentration and/or Duration Parameter by Age

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1	Animal Species Route of Exposure	--		411.5931
2	Animal Species Route of Exposure Age	--	--	402.3871
3	Animal Species Route of Exposure	Age	--	403.5792
4	Animal Species Route of Exposure	--	Age	400.8103
5	Animal Species Route of Exposure Age	--	Age	402.8616
6	Age Route of Exposure Animal Species	Age	--	404.3548
7	Route of Exposure Animal Species	Age	Age	402.6922
8	Route of Exposure Animal Species Age	Age	Age	404.2105

Cumulative odds model is used in all eight models with the logit link function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) transformed (log10).

Model 4 is associated with the lowest AIC. The effect of age on the duration parameter, the effect of animal species on the intercept and the effect of the route of exposure on the intercept is significant (Table 4.16). The difference between the deviances for models 1 and 4 were tested against a chi-square distribution with two degrees of freedom ($df_{model4} = 9$, $df_{model1} = 7$). The difference between the deviances of the two models (387.5931-374.8103=12.7828) is significant ($p=0.0017$, $df = 2$). In further analyses the

intercept will be stratified by the animal species and the route of exposure and the duration parameter will be stratified by age.

Table 4.16: Model 4 – Test for Effect of Animal Species and Route of Exposure on the Intercept and Age on the Duration Parameter

Stratifications Options in Model 4	*Chi-square*	*df*	*P-value*
Duration Stratified by Age	11.1092	1	0.0009
Intercept Stratified by Route of Exposure[a]	27.6140	3	<0.0001
Intercept Stratified by Animal Species[b]	23.6786	4	<0.0009

Cumulative odds model is used with the logit link function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) are transformed (log10).
[a]Controlling for animal species
[b]Controlling for the route of exposure

To look at the effect of sex, three categories were created including 'males', 'females' and 'both'. Table 4.17 presents the AIC for four models where the intercept, concentration, and/or duration parameter was stratified by sex. In every model, the intercept and concentration parameters are stratified by route of exposure and animal species and the duration parameter is stratified by age.

Table 4.17: AIC Comparison of Four Modeling Options Defined by the Stratification of the Intercept, Concentration or Duration Parameter by Sex (Both, Males & Females)

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1	Route of Exposure Animal Species	--	Age	400.8103
2	Route of Exposure Animal Species Sex	--	Age	400.0694
3	Route of Exposure Animal Species	Sex	Age	404.0913
4	Route of Exposure Animal Species		Sex Age	406.4128

Cumulative odds model is used in all four models with the logit link function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) are transformed (log10).

Model 2 is associated with the lowest AIC. Sex does have an effect on the intercept after controlling for route of exposure and the animal species ($p<0.0001$, $df=9$); however,

there is almost no change in the AIC between this more complex model and the simplified model (Model 1). A model that becomes too complex can result in extremely wide confidence intervals in some categories. Small differences in estimates between genders may only reflect differences in study design rather than specific gender effects.

Another approach to looking at the effect of sex in the model is to remove all the studies that did not report their findings separately for males and females. When all observations categorized as 'both' are removed, the number of observations decreases from to 209 to 183. Using a two-level sex variable (i.e., male and female) the AIC does not decrease when the intercept, concentration and duration parameters are stratified by sex (Table 4.18). The final model will not stratify any parameters by sex.

Table 4.18: AIC Comparison of Four Models Defined by the Stratification of the Intercept, Concentration or Duration Parameter by Sex (Males & Females)

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1	Route of Exposure Animal Species	--	Age	305.0813
2	Route of Exposure Animal Species Sex		Age	311.8269
3	Route of Exposure Animal Species	Sex	Age	306.5146
4	Route of Exposure Animal Species		Age Sex	307.0812

Cumulative odds model is used in all four models with the logit link function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) are transformed (log10).

The final cumulative odds model selects the logit link function, transforms concentration and duration (log 10), stratifies the intercept by animal species and route of exposure and stratifies duration by age. Table 4.19, which presents the parameters in the exposure-response curve, their standard errors and significance level associated with the null

hypothesis that the true value of the parameter is zero, indicates that each coefficient estimate is statistically different from zero.

Table 4.19: Parameter Estimates, Standard Errors, Z-test Statistics and P-values for Copper Excess Studies Using the Cumulative Odds Model*

Parameter	*Estimate*	*Std. Error*	*Z-test*	*P-value*
SEV1	-2.7849	1.0365	-2.6858	0.0072
SEV2	-3.2931	1.0561	-3.1183	0.0018
SEV3	-3.5636	1.0509	-3.3911	0.0007
SEV4	-4.5955	1.0364	-4.4341	<0.0001
SEV5	-9.2695	1.2214	-7.5896	<0.0001
SEV6	-9.8075	1.2553	-7.8129	<0.0001
HU:F:INTERCEPT	0	0	NA	NA
HU:W:INTERCEPT	1.7650	0.7737	2.2812	0.0225
MU:F:INTERCEPT	-7.9434	1.7623	-4.5073	<0.0001
MU:W:INTERCEPT	-3.5169	1.6529	-2.1278	0.0334
RT:F:INTERCEPT	-6.8773	1.6423	-4.1741	<0.0001
RT:W:INTERCEPT	-4.9720	1.5968	-3.1136	0.0019
LG10CONC	3.7761	0.5911	6.3880	<0.0001
1:LG10TIME	3.3906	0.7540	4.4969	<0.0001
2:LG10TIME	2.3576	0.6084	3.8750	<0.0001

* Cumulative odds model uses the logit link function. Concentration (mg/kg $bw^{2/3}$/days) and duration (days) have been log transformed to the base 10. Note. SEV, severity level; LG10, log transformed to the base 10; CONC, concentration coefficient; TIME, duration coefficient; HU, humans; RT, rats; MU, mice; F, dietary studies; W, drinking water studies; 1, young animal (≤30 days of age); 2, mature animal (>30 days of age for rodents and ≥18 years for humans).

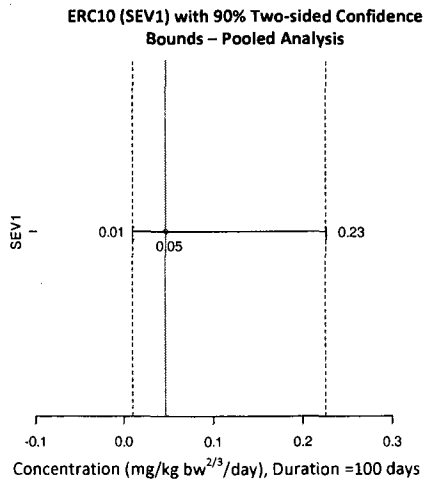
Once the model has been stratified, ERC10-T100 estimates can be produced for each stratum in the model. The ERC10-T100 corresponds to the exposure concentration at which the probability is 0.10 of an adverse effect of level s or higher occurring due to an exposure of 100 days in duration. Comparing the ERC10-T100 estimates in the stratified analysis can help determine whether there are practical differences within each stratified variable.

Figures 4.3a-l present the ERC10-T100 estimates for the pooled analysis (no stratified parameters) and the stratified analysis for each severity level. There are major differences between the ERC10 estimates in the stratified analysis. For severity levels 1 to 4, the human estimates in the stratified analysis are more similar to the estimates in the pooled analysis, compared to other animal species. Compared to the pooled estimate, the stratified analysis reduces the width of the confidence intervals around the human ERC10 estimates.

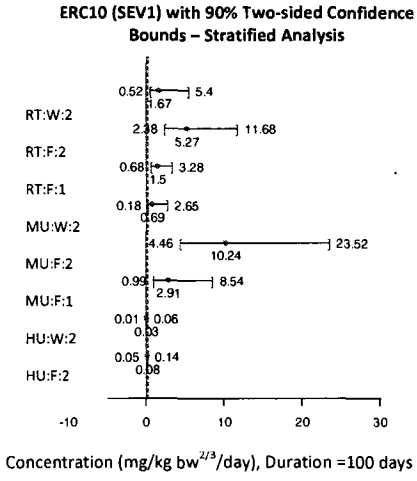
By separating out the systematic differences in animal species, route of exposure, and age, the model becomes more focused and accounts for a large portion of the variation in the copper database. For some of the strata in Figure 4.3, the confidence interval on the ERC10 estimates are quite large, especially for dietary studies on rats and mice. When the model is stratified by the animal species, route of exposure, and age, there are only two observations in the weanling mice stratum. For the mature mice dietary stratum, there are no observations at severity levels 1 to 3 or at severity levels 5 and 6 and there are only two different exposure durations.

As stratification increases, we should expect to see some confidence intervals increase as there is less data available in each category. However, for rats and mice, the large difference in the estimates between dietary and water studies and between age categories for the dietary studies emphasize the need to stratify by age and route of exposure. The ERC10 estimates for humans appear to be associated with more narrow confidence intervals than the ERC10 estimates for animals. This is due largely to the fact that the estimates for humans are several magnitudes lower than the estimates for animals. This will be discussed further in Part 5.

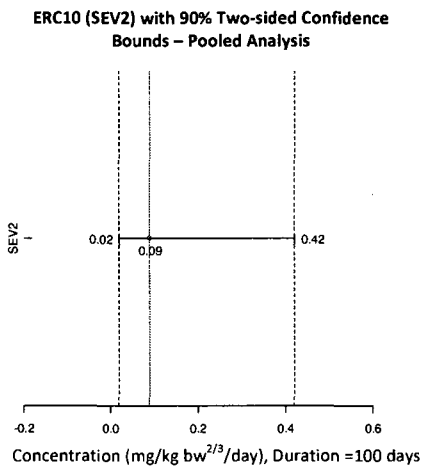
a)



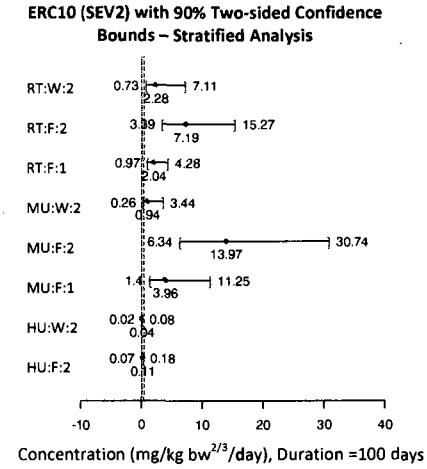
b)



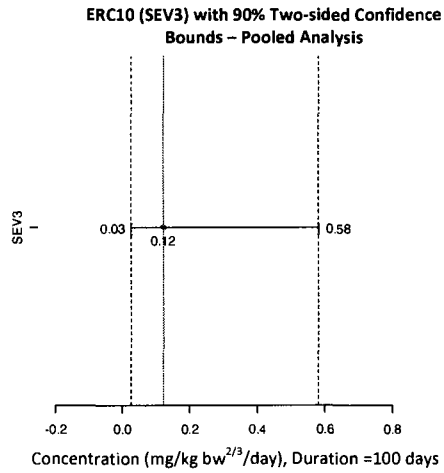
c)


ERC10 (SEV2) with 90% Two-sided Confidence Bounds – Pooled Analysis
SEV2
0.02
0.09
0.42
-0.2
0.0
0.2
0.4
0.6
Concentration (mg/kg bw2/3/day), Duration =100 days

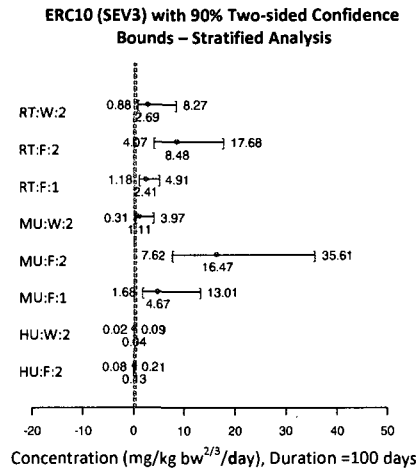
d)


ERC10 (SEV2) with 90% Two-sided Confidence Bounds – Stratified Analysis
RT:W:2
RT:F:2
RT:F:1
MU:W:2
MU:F:2
MU:F:1
HU:W:2
HU:F:2
-10
0
10
20
30
40
Concentration (mg/kg bw2/3/day), Duration =100 days

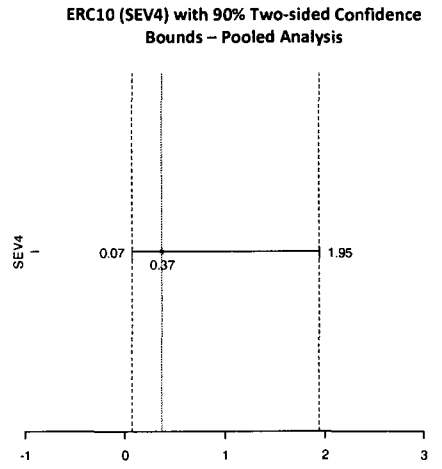
e)


ERC10 (SEV3) with 90% Two-sided Confidence Bounds – Pooled Analysis
SEV3
0.03
0.12
0.58
-0.2
0.0
0.2
0.4
0.6
0.8
Concentration (mg/kg bw2/3/day), Duration =100 days

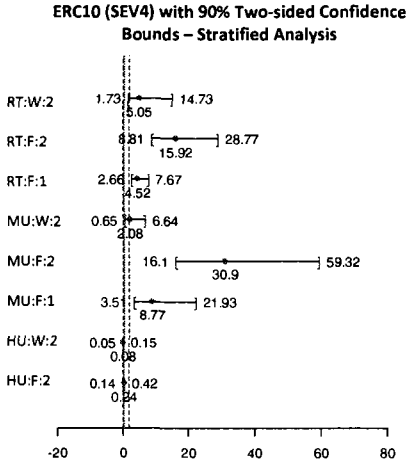
f)


ERC10 (SEV3) with 90% Two-sided Confidence Bounds – Stratified Analysis
RT:W:2
RT:F:2
RT:F:1
MU:W:2
MU:F:2
MU:F:1
HU:W:2
HU:F:2
-20
-10
0
10
20
30
40
50
Concentration (mg/kg bw2/3/day), Duration =100 days

g)


ERC10 (SEV4) with 90% Two-sided Confidence Bounds – Pooled Analysis
SEV4
0.07
0.37
1.95
-1
0
1
2
3
Concentration (mg/kg bw2/3/day), Duration =100 days

h)


ERC10 (SEV4) with 90% Two-sided Confidence Bounds – Stratified Analysis
RT:W:2
RT:F:2
RT:F:1
MU:W:2
MU:F:2
MU:F:1
HU:W:2
HU:F:2
-20
0
20
40
60
80
Concentration (mg/kg bw2/3/day), Duration =100 days

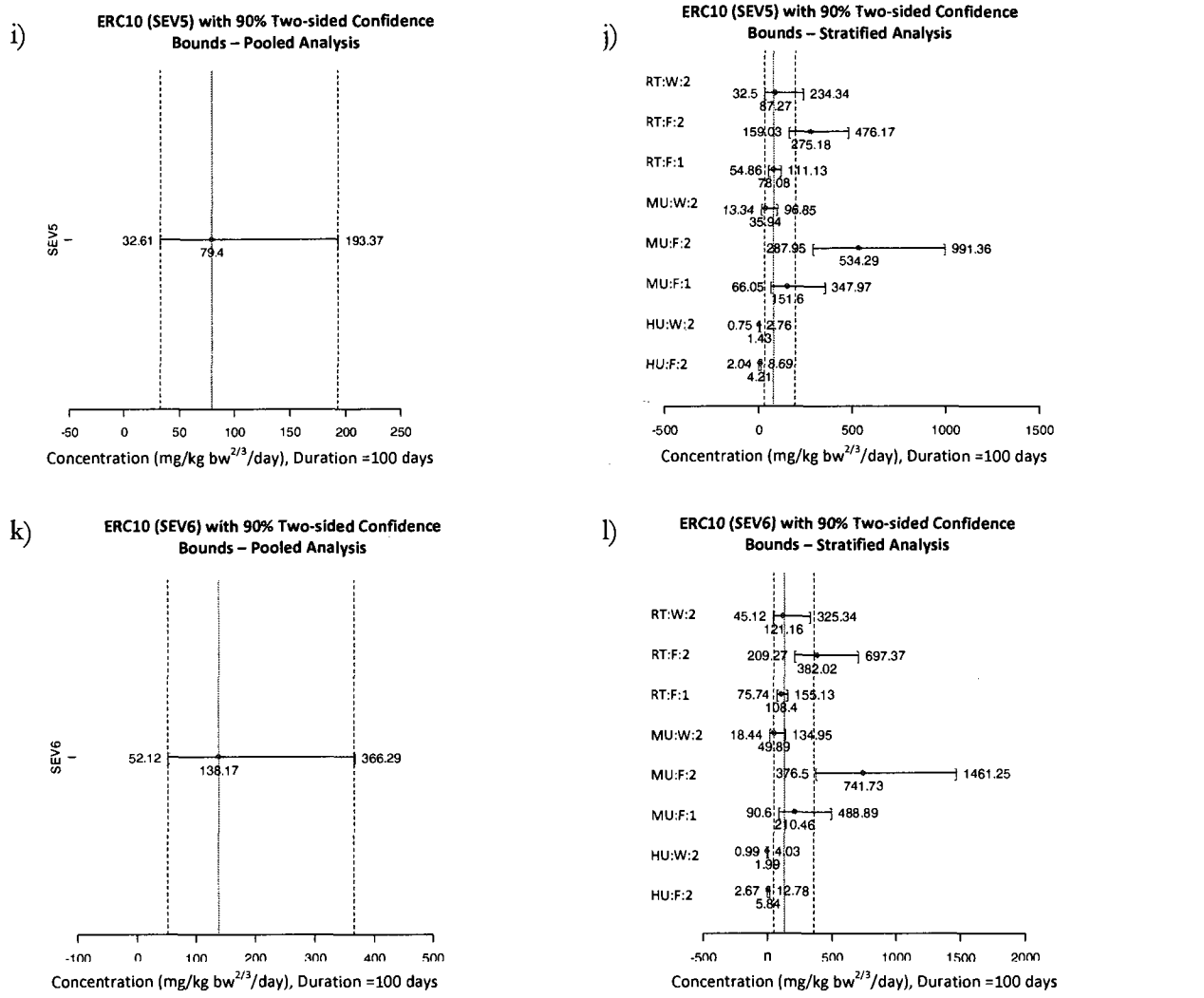


Figure 4.3a-l: Figures 4.3 a, c, e, g, i and k represent the ERC10 estimates with 90% two-sided confidence bounds for a pooled analysis for severity levels 1-6 (SEV1-SEV6), respectively and Figures 4.3 b, d, f, h and j represent the ERC10 estimates with 90% two-sided confidence intervals for a stratified analysis for severity levels 1-6, respectively. Note. HU, humans; RT, rats; MU, mice; F, dietary studies; W, drinking water studies; 1, young animal (≤30 days of age); 2, mature animal (>30 days of age for rodents and ≥18 years for humans).

4.5B Sensitivity Analyses

The biological rational for not including acute exposure studies was reviewed in Part 3. As expected, adding the acute exposure studies increases the magnitude of the standard errors relative to the size of the parameter estimates. Table 4.20 presents the parameter estimates, their standard errors, Z-test statistics and p-values for the analysis with the acute exposure studies. Parameter estimates for severity levels 1 to 4, the coefficient for the mice drinking water stratum, and the coefficients for duration are no longer statistically significant.

Further background on acute exposure studies and their impact on the analysis will be discussed in Part 5. Acute exposure studies will continue to be excluded from the categorical regression analysis with the cumulative odds model.

Table 4.20: Parameter Estimates, Standard Errors, Z-test Statistics and P-values using the Cumulative Odds Model* - Including Acute Exposure Studies

Parameter	*Estimate*	*Std. Error*	*Z-test*	*P-value*
SEV1	0.2445	0.9772	0.2502	0.8025
SEV2	-0.1254	0.9828	-0.1276	0.8984
SEV3	-0.1319	0.9862	-0.3238	0.7461
SEV4	-1.0586	1.0421	-1.0159	0.3097
SEV5	-5.3307	0.9315	-5.7226	<0.0001
SEV6	-5.7780	0.9242	-6.2518	<0.0001
HU:F:INTERCEPT	0	0	NA	NA
HU:W:INTERCEPT	3.0002	0.8620	3.4805	0.0005
MU:F:INTERCEPT	-4.9088	1.3676	-3.5894	0.0003
MU:W:INTERCEPT	-1.4538	1.5095	-0.9631	0.3355
RT:F:INTERCEPT	-3.9934	1.2363	-3.2302	0.0012
RT:W:INTERCEPT	-3.0905	1.2994	-2.3784	0.0174
LG10CONC	2.5442	0.4110	6.1911	<0.0001
1:LG10TIME	0.8653	0.4685	1.8470	0.0648
2:LG10TIME	0.0071	0.4287	0.0164	0.9869

* Cumulative odds model uses the logit link function. Concentration (mg/kg $bw^{2/3}$/days) and duration (days) have been log transformed to the base 10. Note. SEV, severity level; LG10, log transformed to the base 10; CONC, concentration coefficient; TIME, duration coefficient; HU, humans; RT, rats; MU, mice; F, dietary studies; W, drinking water studies; 1, young animal (≤30 days of age); 2, mature animal (>30 days of age for rodents and ≥18 years for humans).

Issues with the human drinking water studies were discussed in section 4.3E. The available drinking water studies using subacute and subchronic durations of exposure have only used doses that have been associated with severity level 0 (no effect) and severity level 4. As all responses assigned to severity level 4 were gastrointestinal symptoms from elevated copper intake, severity level 4 may only represent an acute response to copper in drinking water and not a long term consequence of elevated intake. All observations from human studies that utilized drinking water as the route of exposure were excluded from the analysis. Table 4.21 presents the results of the ERC10-T100 with and without the human drinking water studies for severity levels 1 to 4. There is negligible change in the ERC10-T100 estimates at severity levels 1 to 3 and only a very small increase (less than 0.09 mg/kg $bw^{2/3}$/day) in the ERC10-T100 at severity level 4. There does appear to be a large difference

(approximately 17% change) in the ERC10 estimates for severity levels 5 and 6 in the models with and without the drinking water studies. As there is no dietary data from human studies that have found responses at severity level 4, when the human drinking water studies are removed, estimates for severity levels 4 to 6 must rely only on data from animal studies. Drinking water studies will be kept in the analysis as their addition results in more precautionary estimates for the ERC10 at severity levels 5 and 6 and they appear to have minimal impact on the ERC10 for lower levels of severity.

Table 4.21: ERC10-T100 and Two-sided 90% Confidence Intervals (CI) for the Human Dietary Stratum – With and Without Human Drinking Water Studies

Model #	ERC10-T100 (90% CI)					
	SEV 1	***SEV 2***	***SEV 3***	***SEV 4***	***SEV 5***	***SEV 6***
1[a]	0.08 (0.05, 0.14)	0.11 (0.07, 0.18)	0.13 (0.08, 0.21)	0.24 (0.14, 0.42)	4.21 (2.04, 8.69)	5.84 (2.67, 12.78)
2[b]	0.08 (0.04, 0.14)	0.11 (0.07, 0.19)	0.14 (0.09, 0.23)	0.33 (0.19, 0.58)	5.04 (2.39, 10.65)	7.05 (3.15, 15.79)

[a]Model 1 includes human drinking water studies.
[b]Model 2 does not include human drinking water studies.
Note. SEV, severity level.

A series of studies were identified in section 4.2 that utilized a less soluble form of copper. Only one study was identified among the copper excess studies. This study was on weanling rats exposed to excess levels of copper in their diet (Liu et al., 1986). If this study had an effect on the analysis, we might see its removal increase the ERC10 for weanling rats as less soluble forms of copper may be less bioavailable than more soluble forms of copper. We have defined the dose by the daily amount of copper consumed and not the amount absorbed. For example, suppose that 5 mg of copper was consumed in two different experiments, but in experiment A using a less soluble form of copper resulted in no effects on the outcomes of interest, and in experiment B using a more soluble form of copper resulted in severe responses. Experiment A would underestimate the risk of adverse health effects at 5 mg/day when using a more soluble form of copper due to the decreased proportion of the total amount of copper absorbed in the gastro intestinal tract. Table 4.22

presents the ERC10-T100 for the weanling rat dietary stratum with and without this study. There is a minimal change (less than 4%) in the ERC10-T100.

Table 4.22: ERC10-T100 and Two-sided 90% Confidence Intervals (CI) for the Weanling Rat Dietary Stratum – Effect of Solubility

Analysis	*ERC10-T100 (90% CI)*
With Liu et al. (1986)	2.04 (0.97, 4.28)
Without Liu et al. (1986)	2.12 (0.93, 4.83)

4.5C Data Review for Outliners

CatReg was used to generate a plot of the generalized deviance residuals versus the observation number. As CatReg is unable to generate a deviance plot for a model with more than five strata, two deviance plots were used. Figure 4.4 plots the data for the human and mice strata and Figure 4.5 plots the data for the rat and human strata. Between Figure 4.4 and Figure 4.5 all observations for the eight strata are plotted.

There does not appear to be any one stratum that is poorly described by the exposure-response curve. The observation with the largest deviance, which is found in Figure 4.5, falls within the mature rat dietary stratum. This data point corresponds with one of the observations from Murthy's study (1981). A severity level 3 was assigned to several endpoints including increased dopamine, norepinephrine, and 5-hydroxytrytptamine levels in the brain. This particular study used a dose of 250 mg Cu/kg of feed. Other studies in the analysis have used longer durations and higher levels of copper and have not found responses associated with as severe a severity score. Nervous system functioning appears to be a sensitive endpoint; therefore, the inclusion of this observation from the study by Murthy et al. (1981) is important for ensuring that the risk of adverse effects at severity level 3 or greater is not underestimated.

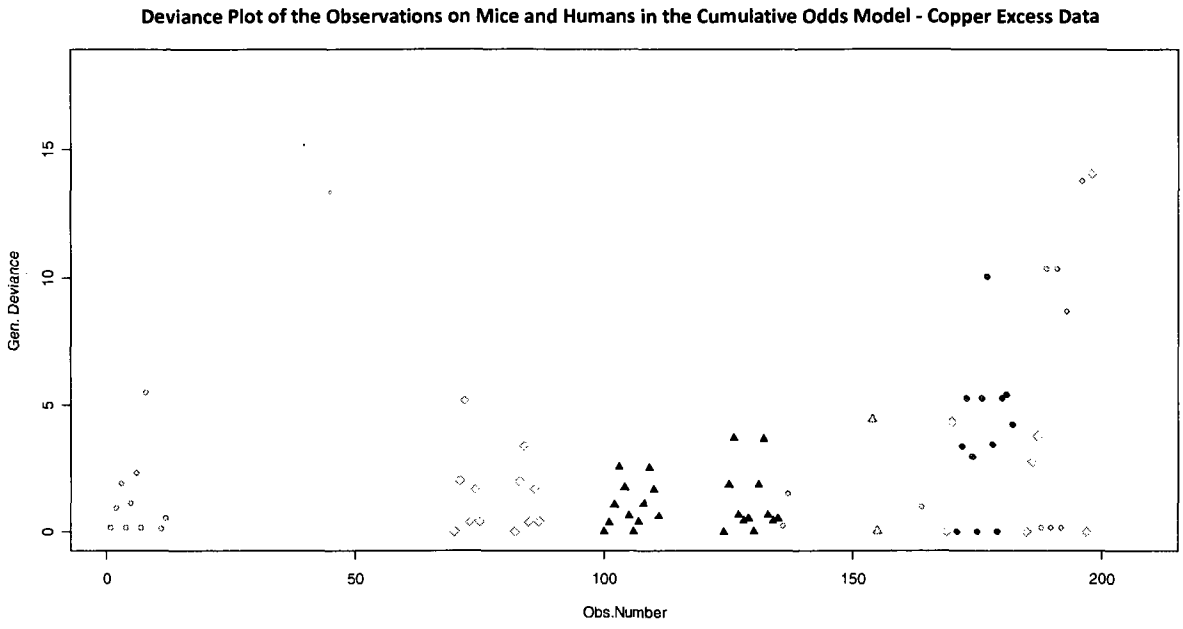


Figure 4.4: Deviance Plot by Observation #. Human Feed Studies = ○, Human Water Studies =●, Young Mice Feed Studies =△, Mature Mice Feed Studies =▲, Mature Mice Drinking Water Studies =◇. Note. Obs, observation number; Gen., general.

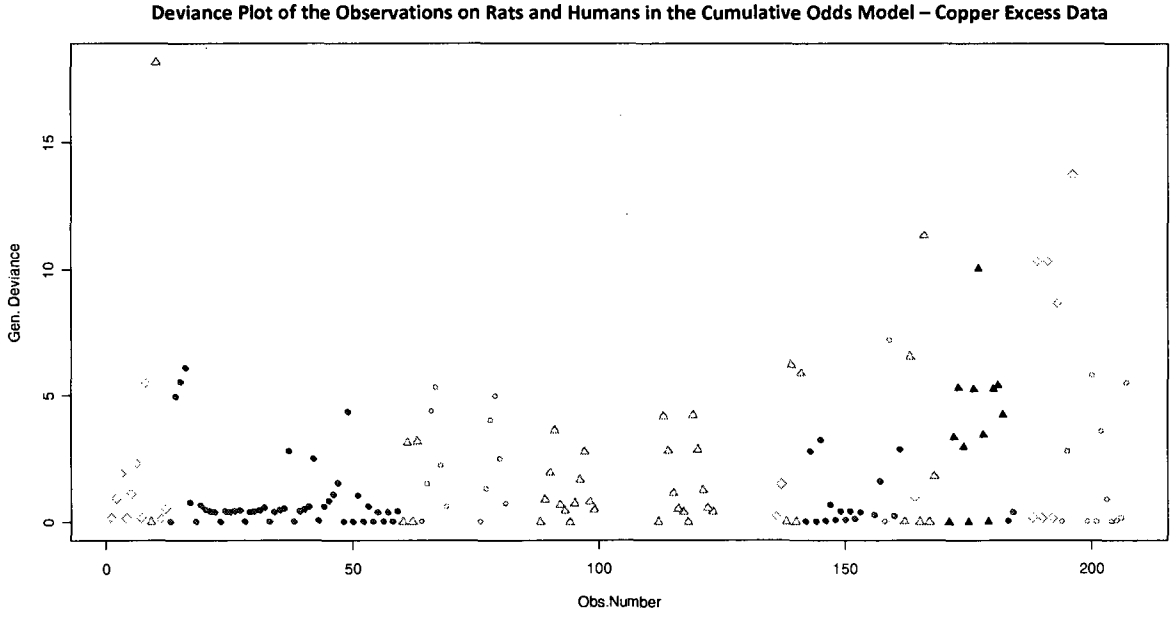


Figure 4.5: Deviance Plot by Observation #. Adult Rat Water Study = ○, Young Rat Feed Studies = ●, Adult Rat Feed Studies =△, Human Water Study =▲, Human Feed Study = ◇. Note. Obs, observation number; Gen., general.

The second highest deviance residual is from the study by Turnlund (2004). This is an important study, as it measured a broad range of copper indices including immune cell functioning, antioxidant status, and other traditional indicators of copper status (e.g., serum copper and ceruloplasmin) not found in other studies.

4.5D Model Fit

In the following section, the cumulative odds model defined in sections 4.5A to 4.5B was used to generate a series of plots for each stratum including a plot of the ERC10 line for severity level 2 or greater with two-sided 90% confidence intervals and a plot of all the ERC10 lines for each severity level. If the model is defined by the animal species, age, and route of exposure there could be 12 different strata in the model; however, as there are no drinking water studies on young animals, and there are no human studies on younger age groups, only eight different strata are available in the exposure-response model. A plot of the ERC10-T100 line for severity level 2 or greater and a plot of the ERC10-T00 for all severity levels is presented below for the human strata only (dietary and drinking water strata). The plots for rats and mice can be found in Appendix G. It is important to note that the ERC10 lines will not run through their corresponding observations. For the ERCq, as q increases from 0 to 1, the ERCq lines should approach their corresponding observations. As we have defined q equal to 0.10, the ERC10 lines for copper excess should fall below all corresponding observations.

Figures 4.6 and 4.7 present a plot of the ERC10 line for severity level 2 or greater with 90% two-sided confidence intervals for the human dietary studies and human drinking water studies, respectively. Figure 4.8 and 4.9 plots the ERC10 lines for all severity levels for the human dietary stratum and the human drinking water stratum, respectively.

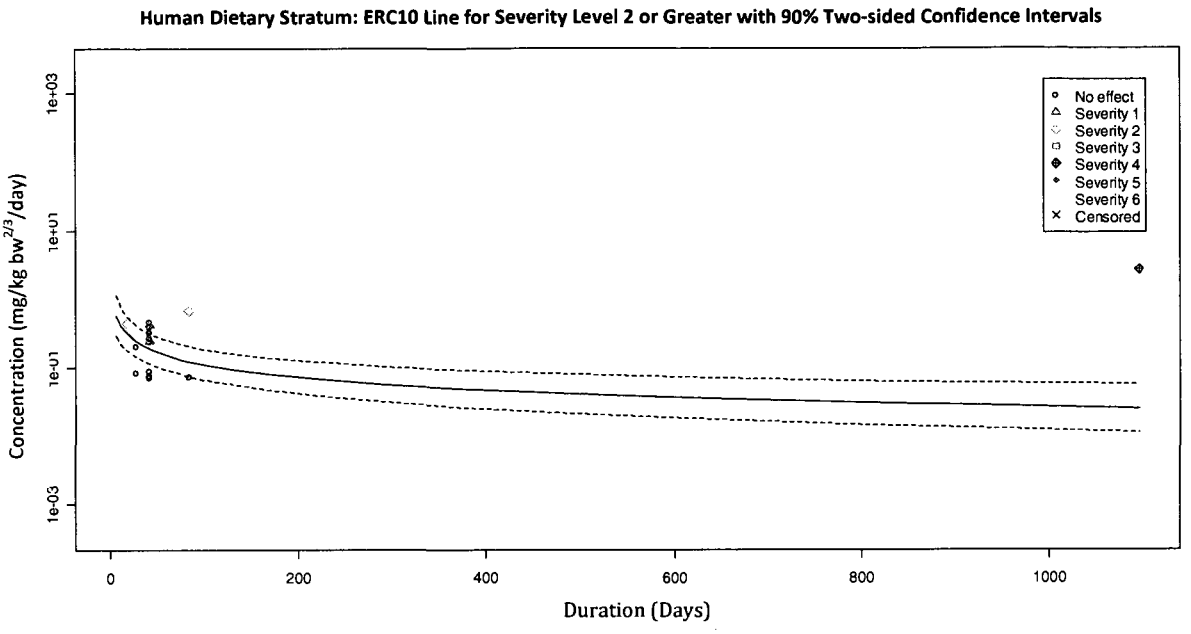


Figure 4.6: Cumulative odds model with the logit link function. Concentration and duration transformed (log10). Intercept stratified by animal species and route of copper exposure. Duration stratified by age.

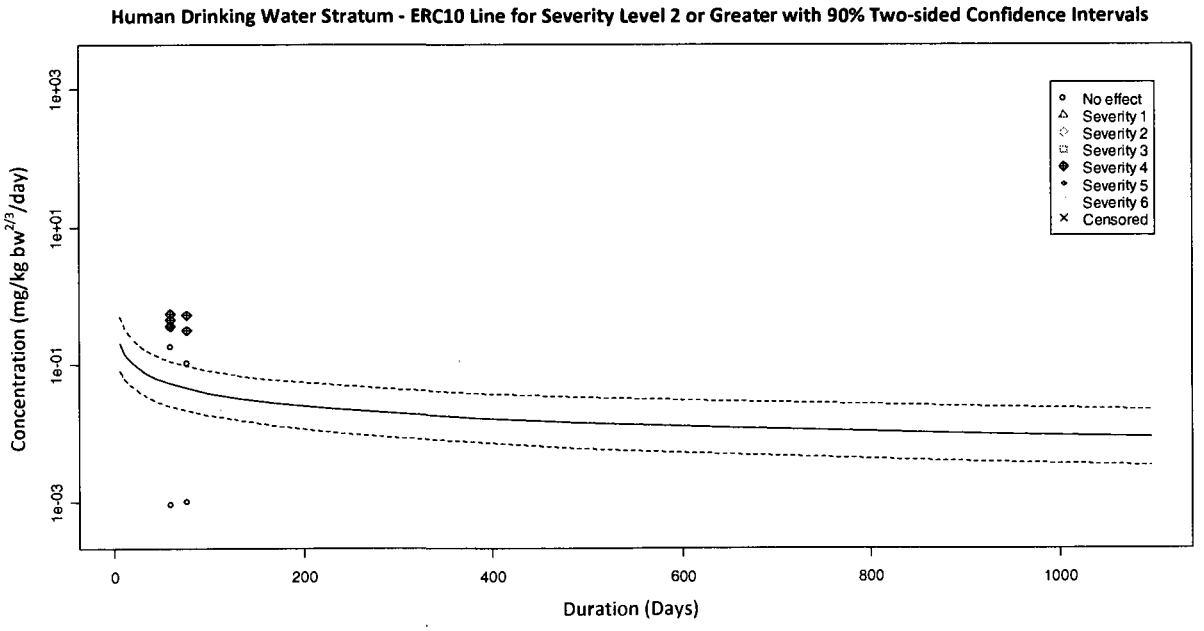


Figure 4.7: Cumulative odds model with the logit link function. Intercept stratified by animal species and route of copper exposure. Duration stratified by age.

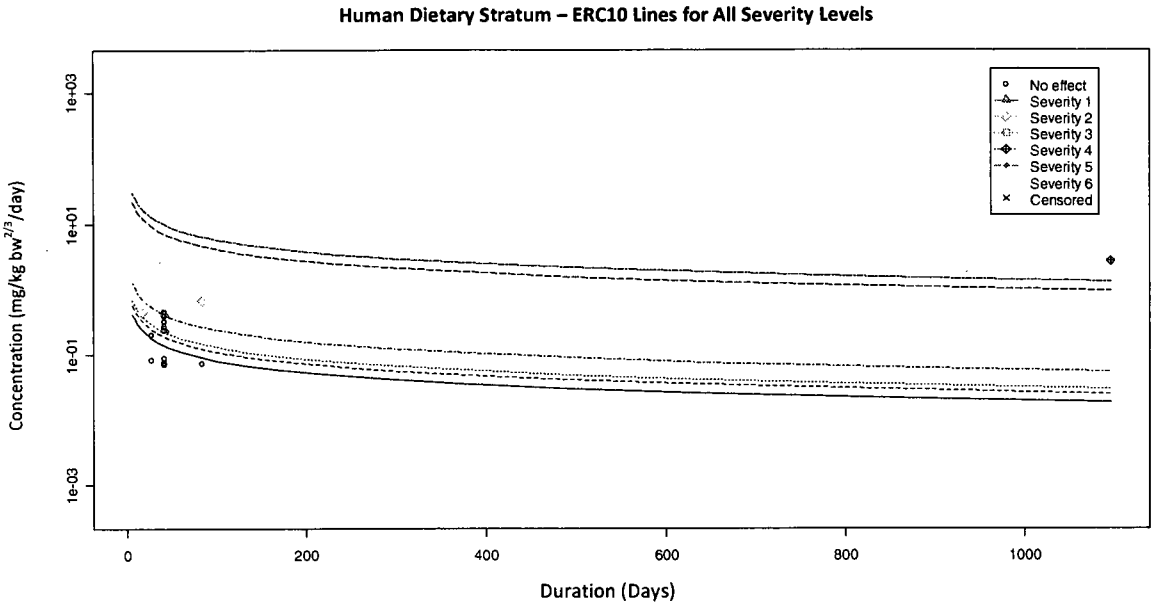


Figure 4.8: Cumulative odds model with the logit link function. Concentration and duration transformed (log10). Intercept stratified by animal species and route of copper exposure. Duration stratified by age.

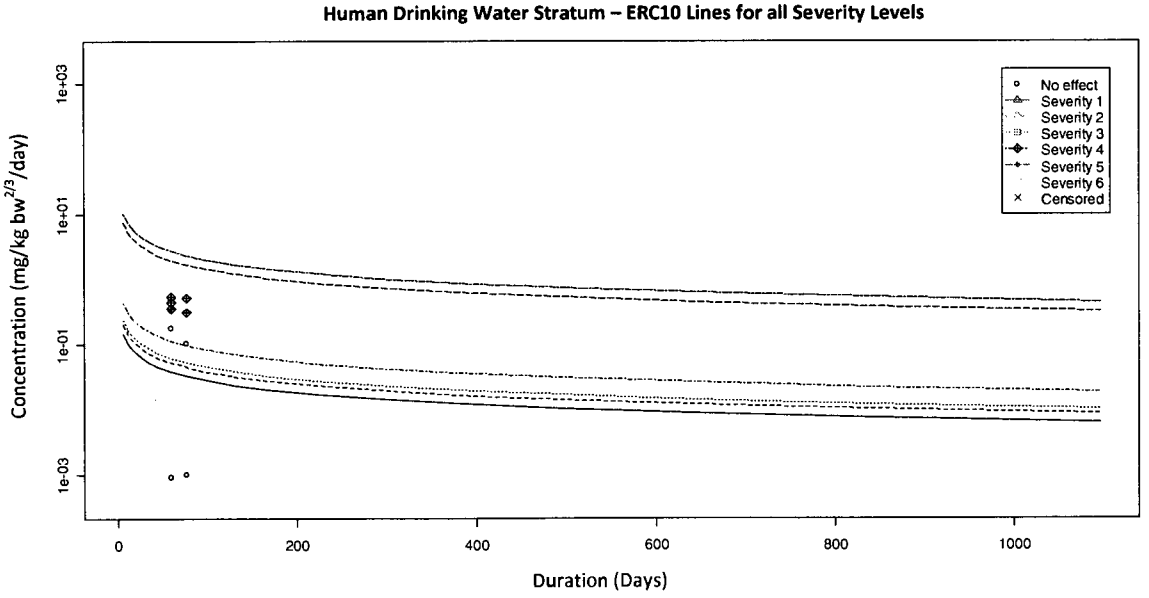


Figure 4.9: Cumulative odds model with the logit link function. Intercept stratified by animal species and route of copper exposure. Duration stratified by age.

As expected, the ERC10 line in all figures has a negative slope, as longer durations require a lower concentration to achieve the estimated 10% response probability. While all of the observations on humans, rats and mice are used to define the exposure-response

model and plot the ERC10 lines, only the observations that correspond to the stratum specific ERC10 plots are presented. As some strata will not have observations available at all severity scores, information from other strata is used to define these ERC10 lines. For example, for the human dietary stratum, the ERC10 lines for severity levels 3 to 6 are an extrapolation outside the range of the available data as there are no observations in this stratum that correspond to these severity levels. In this analysis only the weanling rat dietary stratum has observations at all levels of severity.

Model fit can be evaluated by checking to see if the ERC10 line for each severity level is below its corresponding observations. For example, the ERC10 line for severity level 3 or greater should be below all observations assigned to severity level 3. We cannot, however, evaluate those ERC10 lines that correspond to severity levels where there are no corresponding observations. For example, as there are only observations at severity level 1 and 2 for the human dietary stratum (Figure 4.8), we can only evaluate whether the ERC10 lines for severity levels 1 and 2 are below their corresponding observations. For this stratum the ERC10 lines for severity levels 1 and 2 are below their corresponding observations. For the human drinking water stratum, young rat dietary stratum, mature rat dietary stratum, mature rat drinking water stratum, young mice dietary stratum, and mature mice drinking water stratum, all ERC10 lines are below their corresponding observations. For the adult mice dietary stratum, the gap is very narrow between severity levels 0 and 4 highlighting the need for more studies with mature mice looking at marginally excess levels of copper. In every strata, the plot of all the ERC10 lines shows that there is a large gap between the ERC10 lines for severity level 4 and severity level 5. This large gap between severity levels 4 and 5 will be discussed in Part 5. Only the mature mice drinking water stratum, the mature rat drinking water stratum and the weanling rat dietary stratum have observations associated

with a severity level 5. For those strata that contain observations at severity level 5 the ERC10 line does fall below all observations associated with this severity level.

Variability in observed toxicity is demonstrated by overlap in the assignment of severity scores at common concentrations and durations of exposure. For example, for the human dietary stratum there is some variability between the assignment of severity levels 0 and 1 (Figure 4.6); however, for the drinking water studies on humans (Figure 4.7) there is no variability in the assignment of severity scores. There is some variability in observed toxicity at 7 days of duration in the weanling rat dietary stratum (Figure G2 in Appendix G). For the mature rat drinking water stratum there is some variability in observed toxicity between 7 and 20 days of exposure (Figure G6 in Appendix G). This variability in observed toxicity will be discussed in Part 5.

Overall the cumulative odds model of the copper excess data with the intercept stratified by the animal species and route of exposure and the duration parameter stratified by age appears to fit the data well. If the model did not fit the data well we would expect that some ERC10 lines would not fall fellow their corresponding observations.

Figure 4.10 plots all the ERC10 lines by concentration and duration for each stratum. It is difficult to visualize which ERC10 lines correspond do which strata; however, there are no options in CatReg that will improve the presentation of the plotting options available. At 800 days, the ordering of the curves by their corresponding strata from top to bottom are: mature mice dietary stratum, mature rat dietary stratum, mature rat drinking water stratum, weanling mice dietary stratum, mature mice drinking water stratum, weanling mice dietary stratum, adult human dietary stratum and the adult human drinking water stratum. Figure 4.10 demonstrates that the animal species, route of exposure and age will impact how the exposure-response curve for copper excess is characterized. For example, the slope for the

weanling rat and mice stratum is greater than the slope for the mature rat and mice stratum. Holding age and route of exposure constant, the impact of the animal species has a large impact on the intercept but not the slopes of the exposure-response curve. The same is true for the route of exposure. As the model's intercept is the only parameter that has been stratified by the route of exposure, the difference in the ERC10 lines for the dietary and drinking water strata for each animal species remains constant as duration increases. After 150 days the data becomes very sparse. The aim of this analysis is to define estimates for long-term chronic exposures to copper; however, there is only one data point after 100 days of exposure for the human stratum. Duration of exposure at 100 days is considered a chronic exposure for rats and mice but only a subchronic exposure for humans. In order to define the ERC for a long-term exposures and stay within the limits of the data, all further ERC10 estimates will be defined at 100 days.

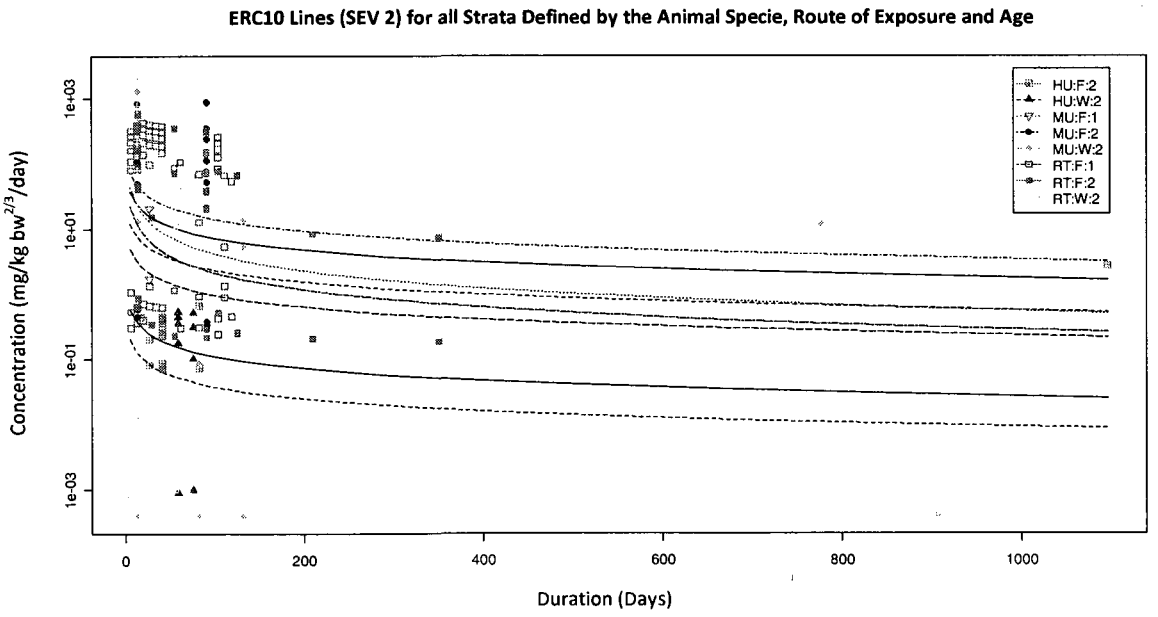


Figure 4.10: Cumulative odds model with the logit link function. Intercept stratified by animal species and route of copper exposure. Duration stratified by age.

4.5E Final Estimates

Table 4.23 presents the ERC10-T100 estimates for severity level 2 or greater by specie, age, and route of exposure. After accounting for interspecies differences in sensitivity based on surface area, the ERC10-T100 for dietary studies is 65.6 times greater for rats than humans and 128.4 times greater for mice than humans. The ERC10 for dietary studies is approximately two times greater for mice than rats. The large difference in the ERC10 estimates between animal species will be discussed in Part 5.

Table 4.24 presents the ERC10 for 25, 50 and 100 days of duration for each stratum. As duration is not stratified by the animal species, the impact of duration is the same for rats, mice, and humans. Had duration not been included in the exposure-response model, the ERC10-T100 estimate for the human dietary stratum would increase slightly from 0.1083 (0.0653, 0.1797) to 0.1191 (0.0720, 0.1970) mg/kg $bw^{2/3}$/day. The impact of duration in the exposure-response model will be discussed further in part 5.

In this categorical regression analysis of the copper excess data, the acceptable range of oral intake (AROI) was thought of as being defined by the ERC10 at severity level 2 or greater. Severity level 2 is assigned to responses that are associated with early responses to accumulated or deficient levels of copper. Based on the cumulative odds model defined in this section, the AROI for humans would be 0.11 mg/kg $bw^{2/3}$/day or 1.88 mg Cu/day assuming an average body weight of 70kg. Part 5 will present the probability curves for severity level 2 or greater for both copper excess and deficiency. The ERC10-T100 estimate for human drinking water studies at severity level 2 or greater is 0.04 mg/kg $bw^{2/3}$/day or 0.68 mg/day. It is important to note that the dose information for drinking water studies in the copper toxicity database does not include background levels of copper in the diet. If we assume that the background diet provides 1.25 mg Cu/day (Baker 1999a) than the AROI

would be 1.93 mg Cu/day. There is little difference in the AROI between the dietary and drinking water studies for humans. In animal studies the difference between the ERC10-T100 at severity level 2 or greater between the dietary and drinking water stratum is much larger. This will be discussed further in Part 5.

Table 4.23: ERC10-T100 with Two-sided 90% Confidence Intervals (CI) for Severity level 2 or Greater by Animal Species, Age and Route of Exposure

Data Included in the Analysis	*ERC10-T100 (90% CI)*
Humans, Dietary Studies	0.11 (0.07, 0.18)
Humans, Water Studies	0.04 (0.02, 0.08)
Mature Rats, Dietary Studies	7.22 (3.35, 15.55)
Weanling Rats, Dietary Studies	2.12 (0.93, 4.84)
Mature Rats, Drinking water Studies	2.31 (0.73, 7.36)
Mature Mice, Dietary Studies	14.12 (6.28, 31.74)
Weanling Mice, Dietary Studies	4.14 (1.35, 12.72)
Mature Mice, Drinking water Studies	0.95 (0.25, 3.60)

Cumulative odds model uses the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) is log transformed to the base 10.

Table 4.24: ERC10 Estimates with Two-sided 90% Confidence Intervals for Severity Level 2 or Greater at an Exposure Duration (T) of 25, 50 and 100 Days

Data Included in the Analysis	ERC10-T100 (90% CI)		
	T=25	*T=50*	*T=100*
Humans, Dietary Studies	0.22 (0.13, 0.35)	0.17 (0.10, 0.28)	0.11 (0.07, 0.18)
Humans, Water Studies	0.07 (0.04, 0.14)	0.06 (0.03, 0.12)	0.04 (0.02, 0.08)
Mature Rat, Dietary Studies	14.15 (7.50, 26.67)	11.09 (5.20, 23.65)	7.22 (3.35, 15.55)
Weanling Rat, Dietary Studies	4.15 (2.12, 8.13)	3.92 (1.81, 8.47)	2.12 (0.93, 4.84)
Mature Rat, Water Studies	4.53 (1.55, 13.27)	3.55 (1.15, 10.96)	2.31 (0.73, 7.36)
Mature Mice, Dietary Studies	27.67 (13.70, 55.86)	21.70 (9.94, 47.37)	4.12 (6.28, 31.74)
Weanling Mice, Dietary Studies	8.12 (2.91, 22.67)	7.66 (2.73, 21.49)	4.14 (1.35, 12.72)
Mature Mice, Water Studies	1.86 (0.56, 6.19	1.46 (0.20, 5.32)	0.95 (0.25, 3.60)

Cumulative odds model uses the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) is log transformed to the base 10.

4.6 UNRESTRICTED CUMULATIVE MODEL – COPPER EXCESS

Section 4.5 compared a series of modeling options with the cumulative odds model of the copper excess data. In the cumulative odds model, the ERCq lines for each severity level will be parallel. Unlike the cumulative odds model, the unrestricted cumulative model does not assume that the ERCq lines are parallel as separate coefficients for concentration and duration are estimated for each severity level (Equation 2) (US EPA, 2000). Modeling the data with this more complex model allows us to evaluate whether the similar model (the

cumulative odds model) is adequate to describe the exposure-response data. Section 4.6 repeats the steps outlined in section 4.5 using the unrestricted cumulative model. Allowing the concentration and duration parameter to vary by the level of severity may change which stratification options have a significant effect in the exposure-response model.

4.6A Severity Score Combinations

The unrestricted cumulative model is more complex than the cumulative odds model as more parameters need to be estimated. Fitting the unrestricted cumulative model to the copper excess data produces an error message in CatReg, indicating there is a need to simplify the model due to incorrectly ordered severity estimates. Table 4.25 presents the estimates, standard errors, z-test statistics, and p-values for each parameter in the model. Incorrectly ordered severity parameter estimates may suggest that there are too many severity levels in the data (US EPA, 2000).

Table 4.25: Parameter Estimates, Standard Errors, Z-test Statistics and P-values for Copper Excess Studies Using the Unrestricted Cumulative Model*

Parameter	*Estimate*	*Std. Error*	*Z-test*	*P-value*
SEV1	-15.0080	3.2249	-4.6539	<0.0001
SEV2	-3.9056	1.0485	-3.7251	<0.0001
SEV3	2.5479	1.2620	2.0191	0.0435
SEV4	0.7314	1.4200	0.5151	0.6065
SEV5	-11.6435	2.5464	-4.5725	<0.0001
SEV6	10.0464	11.6664	0.8611	0.3892
LG10CONC:SEV1	3.8330	0.8364	4.5825	<0.0001
LG10TIME:SEV1	6.4218	1.3766	4.6649	<0.0001
LG10CONC:SEV2	-1.2479	0.2691	-4.6375	<0.0001
LG10TIME:SEV2	4.0309	0.7281	5.5265	<0.0001
LG10CONC:SEV3	0.8979	0.3501	2.5646	0.0103
LG10TIME:SEV3	-1.2175	0.6900	-1.7645	0.0777
LG10CONC:SEV4	0.5989	0.3484	1.7188	0.0857
LG10TIME:SEV4	-0.5874	0.6397	-0.9183	0.33584
LG10CONC:SEV5	3.1423	0.8504	3.6950	0.0002
LG10TIME:SEV5	-0.44344	2.3437	-0.1892	0.8499
LG10CONC:SEV6	1.0473	0.7763	1.3491	0.1773
LG10TIME:SEV6	-7.1728	6.7259	-1.0665	0.2862

* Unrestricted cumulative model with the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed.
Note. SEV, severity level; LG, logarithm; CONC, concentration; TIME, exposure duration.

A series of severity score combinations are presented in Appendix H. A three-level severity model was the only combination that resulted in correctly ordered parameter estimates for the severity scores. Severity scores 0 and 1 were combined to represent level 0; scores 2 to 4 were combined to represent level 1; and scores 5 and 6 were combined to represent level 2. Table 4.26 presents the parameter estimates, standard errors, z-test statistics and p-values for each coefficient in the simplified three-level severity model.

Table 4.26: Parameter Estimates, Standard Errors, Z-test Statistics and P-values for the Unrestricted Cumulative Model* with Three Levels of Severity

Parameter	*Estimate*	*Std. Error*	*Z-test*	*P-value*
SEV1	-5.0815	1.1356	-4.4747	<0.0001
SEV2	-12.9378	3.2586	-3.9704	<0.0001
LG10CONC:SEV1	1.4802	0.2115	6.9973	<0.0001
LG10TIME:SEV1	2.4062	0.6432	3.7407	0.0002
LG10:CONC:SEV2	3.7842	1.3894	2.7237	0.0065
LG10:TIME:SEV2	1.0314	1.3026	0.7918	0.4285

* Model is defined by the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed. Note. SEV, severity level; LG10, base 10 logarithm; CONC, concentration; TIME, exposure duration.

4.6B Assumption of Parallelism

To test whether the simpler model, the cumulative odds model (used in section 4.5) is adequate to describe the data, there is a need to test whether the parameters representing the effects of concentration and duration at each severity level in the unrestricted cumulative model are equal, that is LGCONC10:SEV1 = LGCONC:SEV2 and LGTIME:SEV1=LGTIME:SEV2.

The cumulative odds model assumes that the exposure-response curves for the different severity levels will be parallel. The null hypothesis is that for the unrestricted cumulative model, the dose and duration parameters for severity level 1 are equal to the dose and duration parameters for severity level 2. If the null hypothesis is not rejected, the cumulative odds model is adequate to describe the data.

The results from a Wald-type chi-square test for the equality of the concentration and duration parameter across severity levels 1 and 2 are presented in Table 4.27. The p-value was not significant (p=0.2402,) indicating that it would be more appropriate to use the simpler cumulative odds model. The analysis will continue to look at the unrestricted cumulative model to compare the impact of using the cumulative odds model versus the unrestricted cumulative model on the final results.

Table 4.27: Test for Equality of Concentration and Duration Parameters Across Severity Level 1 and 2

Chi-square	*Df*	*P-value*
2.8529	2	0.2402

Unrestricted cumulative model is used with the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed.

4.6C Model Selection – Stratification Options

Using the unrestricted cumulative model with three levels of severity did not allow any model parameters to be stratified. Errors messages in CatReg indicated that too many parameters were being calculated for the amount of data available. The model could be left unstratified or the number of severity scores could be further reduced to two levels. Guth et al. (1997) has commented on the fact that an important feature of the categorical regression analysis is that the severity categories can be defined to fit the risk assessment application. For the risk assessment of copper toxicity from excess and deficiency, the goal is defining the ERC10 at severity level 2 or greater. A two-level severity model where severity levels 0 and 1 are combined and where severity levels 2 to 4 are combined would still fulfill this goal. In order to ensure that the model is able to account for a large proportion of the variability in the copper database, the three-level severity model will be reduced to two levels. Severity levels 0 and 1 will be combined to form a new severity level 0 and severity levels 2-6 will be combined to form a new severity level 1.

Table 4.28 presents the parameter estimates, standard errors, z-test statistics, and p-values for the three-level severity model, with Table 4.29 providing the same information for the two-level severity model. Whether we use a three-level severity model or a two-level severity model, there is negligible change in the value of the estimate that represents our severity score of interest (severity level 2 or greater) which is represented by SEV1 in tables 4.28 and 4.29.

Table 4.28: Parameter Estimates, Standard Errors, Z-test Statistics and P-values for Unrestricted Cumulative Model* with Three Levels of Severity

Parameter	*Estimate*	*Std. Error*	*Z-test*	*P-value*
SEV1	-5.0815	1.1356	-4.4747	<0.0001
SEV2	-12.9378	3.2586	-3.9704	<0.0001
LG10CONC:SEV1	1.4802	0.2115	6.9973	<0.0001
LG10TIME:SEV1	2.4062	0.6432	3.7407	0.0002
LG10:CONC:SEV2	3.7842	1.3894	2.7237	0.0065
LG10:TIME:SEV2	1.0314	1.3026	0.7918	0.4285

* Unrestricted cumulative model with the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed. Note. SEV, severity level; LG, logarithm; CONC, concentration; TIME, exposure duration.

Table 4.29: Parameter Estimates, Standard Errors, Z-test and P-values for the Two-Level Severity Model*

Parameter	*Estimate*	*Std. Error*	*Z-test=0*	*P-value*
SEV1	-5.0935	1.1444	-4.4506	<0.0001
LG10CONC	1.4883	0.2119	7.0245	<0.0001
LG10TIME	2.4104	0.6466	3.7279	0.0002

* Two-level severity model with the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed. Note. SEV, severity level; LG, logarithm; CONC, concentration; TIME, exposure duration.

The AIC from a series of models were compared to look at the impact of the animal species on the intercept as well as the concentration and duration parameters in the two-level severity model (Table 4.30).

Table 4.30: Effect of the Animal Species on the Intercept, Concentration and/or Duration Parameter - AIC Comparison of Eight Modeling Options

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1	--	--	--	202.3108
2	Animal Species	--	--	172.9211
3		Animal Species		201.2976
4			Animal Species	171.6862
5		Animal Species	Animal Species	159.0306
6	Animal Species	Animal Species		157.8573
7	Animal Species		Animal Species	175.1284
8	Animal Species	Animal Species	Animal Species	156.4323

All two-level severity models use the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed.

Model 8, where the intercept, concentration and duration parameter are stratified by the animal species, is associated with the lowest AIC. The effect of the animal species on the intercept and duration parameter is not significant (Table 4.31). In model 6, which is associated with the second lowest AIC, the effect of the animal species on the intercept and concentration parameter is significant (Table 4.31).

Table 4.31: Test for the Effect of Animal Species in Models 8 and 6

Model # and Stratification Options	*Chi-square*	*df*	*P-value*
Model 8			
Intercept Stratified by Animal Species	4.4842	2	0.1062
Concentration Stratified by Animal Species	17.9489	2	0.0001
Duration Stratified by Animal Species	3.9673	2	0.1376
Model 6			
Intercept Stratified by Animal Species	21.4945	2	<0.0001
Concentration Stratified by Animal Species	11.7806	2	0.0028

All two-level severity models use the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed.

However, when the intercept and concentration parameter are stratified in model 6, the parameter estimate for severity level 1 or greater is not significant (Table 4.32).

Table 4.32: Parameter Estimates, Standard Errors, Z-test and P-values for the Two-Level Model* - Intercept and Concentration Parameters Stratified by the Animal Species

Parameter	*Estimate*	*Std. Error*	*Z-Test=0*	*P-value*
SEV1	0.5250	2.6076	0.2013	0.8404
HU:INTERCEPT	0.0000	0.0000	NA	NA
MU:INTERCEPT	-7.4475	2.1941	-3.3943	0.0007
RT:INTERCEPT	-8.9064	1.9277	-4.6203	<0.0001
HU:LG10CONC	13.4959	3.9366	3.4283	0.0006
MU:LG10CONC	1.6389	0.5965	2.7476	0.0060
RT:LG10CONC	2.8420	0.3870	7.3442	<0.0001
LG10TIME	3.0472	0.8973	3.3961	0.0007

*Two-level severity model with the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) stratified by the animal species. Note. LG10, log transformed to the base 10; CONC, concentration; TIME, duration; HU, human; RT, rat; MU, mice; SEV, severity.

Model 5, where the concentration and duration parameters are stratified by the animal species has the third lowest AIC. The effect of animal species on the concentration and duration parameter is significant (Table 4.33). The difference between the deviances of model 5 and model 2 was tested against a chi-square distribution with 2 degrees of freedom ($df_{model5} = 6$, $df_{model2} = 4$). As the difference between the deviances of the two models (162.9211-145.0306=17.8905) is significant (p=0.0001, df = 2), the more complex model (model 5) will be used. As indicated in Table 4.34, all parameter estimates in the two-level severity model with the concentration and duration parameter stratified by the animal species are significantly different from zero. Further analyses will stratify the concentration and duration parameter by the animal species.

Table 4.33: Model 5 – Test for the Effect of the Animal Species on the Concentration and Duration Parameter

Stratifications Options in Model 5	*Chi-square*	*df*	*P-value*
Concentration Stratified by Animal Species	13.1110	2	0.0014
Duration Stratified by Animal Species	26.0013	2	<0.0001

2-level severity models use the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed.

Table 4.34: Parameter Estimates, Standard Errors, Z-test and P-values for the Two-Level Model* - Concentration and Duration Parameters Stratified by the Animal Species

Parameters	*Estimate*	*Std. Error*	*Z-Test=0*	*P-value*
SEV1	-7.2244	1.8808	-3.8410	0.0001
INTERCEPT	0.0000	0.0000	NA	NA
HU:LG10CONC	13.6215	3.1880	4.2728	<0.0001
MU:LG10 CONC	1.9308	0.7462	2.5876	0.0097
RT:LG10CONC	2.5206	0.3589	7.0224	<0.0001
HU:LG10TIME	7.6002	1.4535	5.2288	<0.0001
MU:LG10TIME	2.7760	1.1472	2.4197	0.0155
RT:LG10TIME	2.6599	0.8906	2.9865	0.0028

*Two level severity model uses the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) stratified by the animal species. Note. HU, human; RT, rat; MU, mice; SEV, severity level; LG10, log transformed to the base 10; CONC, concentration; TIME, duration.

The AIC from a series of models were compared to look at the impact of the route of exposure (dietary studies versus drinking water studies) on the intercept, concentration and duration parameters in the two-level severity model (Table 4.35). In every model the concentration and duration parameter has been stratified by the animal species.

Table 4.35: Effect of Route of Exposure on the Intercept, Concentration and/or Duration Parameter - AIC Comparison of Eight Modeling Options

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1		Animal Species	Animal Species	159.0306
2	Route of Exposure	Animal Species	Animal Species	150.0024
3		Animal Species Route of Exposure	Animal Species	158.3148
4		Animal Species	Animal Species Route of Exposure	145.7016
5	Route of Exposure	Animal Species Route of Exposure	Animal Species	148.8262
6	Route of Exposure	Animal Species	Animal Species Route of Exposure	142.7581
7		Animal Species Route of Exposure	Animal Species Route of Exposure	142.6331
8	Route of Exposure	Animal Species Route of Exposure	Animal Species Route of Exposure	144.6284

All models use the two-level severity model with the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed.

Model 7 is associated with the lowest AIC. The animal species and route of exposure has a significant effect on the concentration and duration parameters (Table 4.36). The difference between the deviances of model 7 and model 4 was tested against a chi-square distribution with 3 degrees of freedom (df_{model7} =12, df_{model4} = 9). The difference between the deviances of the two models (125.7016-116.6331=9.0685) is significant (p=0.0284, df = 3). Further analyses will stratify the concentration and duration parameter by the animal species and the route of exposure.

Table 4.36: Model 7 – Test for the Effect of the Animal Species on the Concentration and Duration Parameter

Model 7 Stratification Options	*Chi-square*	*df*	*P-value*
Concentration Stratified by Animal Species[a]	17.5636	4	0.0015
Concentration Stratified by Route of Exposure[b]	11.7628	3	0.0082
Duration Stratified by Animal Species[a]	31.1710	4	<0.0001
Duration Stratified by Route of Exposure[b]	15.5084	3	0.0014

[a]Controlling for route of exposure
[b]Controlling for animal species

Table 4.37 presents the AIC for eight models where the intercept, concentration and duration parameter were stratified by age. In every model the concentration and duration parameter are stratified by the animal species and the route of exposure.

Table 4.37: Effect of Age on the Intercept, Concentration and/or Duration Parameter - AIC Comparison of Eight Modeling Options

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1		Animal Species Route of Exposure	Animal Species Route of Exposure	142.6331
2	Age	Animal Species Route of Exposure	Animal Species Route of Exposure	143.0484
3		Animal Species Route of Exposure Age	Animal Species Route of Exposure	136.3324
4		Animal Species Route of Exposure	Animal Species Route of Exposure Age	136.9935
5	Age	Animal Species Route of Exposure	Animal Species Route of Exposure	137.904

		Age		
6	Age	Animal Species Route of Exposure	Animal Species Route of Exposure Age	138.9691
7		Animal Species Route of Exposure Age	Animal Species Route of Exposure Age	140.3231
8	Age	Animal Species Route of Exposure Age	Animal Species Route of Exposure Age	139.2139

All models use the two-level severity model with the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed.

Model 3 is associated with the lowest AIC. The results of a Wald-type chi-square test of the null hypothesis that the parameters that have been stratified are equal are presented in Table 4.38. The difference between the AIC of model 3 and model 1 was tested against a chi-square distribution with 2 degrees of freedom (df_{model3} =14, df_{model1} = 12). The difference between the deviances of the two models (116.6331-106.3324=10.3007) is significant (p=0.0058, df = 2). Further analyses will stratify the concentration parameter by animal species, route of exposure, and age and will stratify the duration parameter by animal species and route of exposure.

Table 4.38: Model 3 - Test for the Effect of Age and the Animal Species on the Concentration Parameter and the Effect of the Route of Exposure and Animal Species on the Duration Parameter

Model 3 Stratification Options	*Chi-square*	*df*	*P-value*
Concentration Stratified by Animal Species[a]	35.0368	5	<0.0001
Concentration Stratified by Route of Exposure[b]	18.7606	3	0.0003
Concentration Stratified by Age[c]	18.1193	2	0.0001
Duration Stratified by Animal Species[d]	36.5183	4	<0.0001
Duration Stratified by Route of Exposure[e]	22.3620	3	<0.0001

[a]Controlling for age and route of exposure
[b]Controlling for animal species and age
[c]Controlling for animal species and route of exposure
[d]Controlling for route of exposure
[e]Controlling for animal species

To look at the effect of sex, three categories were defined including 'males', 'females' and 'both'. Stratifying the intercept, concentration or duration parameter by sex did not decrease the AIC (Table 4.39).

Table 4.39: Effect of Sex (Males, Females and Both) on the Intercept, Concentration and/or Duration Parameter - AIC Comparison of Four Modeling Options

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1		Animal Species Route of Exposure Age	Animal Species Route of Exposure	136.3324
2	Sex	Animal Species Route of Exposure Age	Animal Species Route of Exposure	139.4301
3		Animal Species Route of Exposure Age Sex	Animal Species Route of Exposure	145.6995
4		Animal Species Route of Exposure Age	Animal Species Route of Exposure Sex	144.4334

All models use the two-level severity model with the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed.

After removing all the studies that did not report findings for males and females separately, the number of observations decreases from to 209 to 183. Table 4.40 presents the AIC for four models where the intercept and the concentration and duration parameters were stratified by sex (male vs. female). When using a two-level sex variable (i.e. males and females) the AIC does not decrease when any parameters were stratified by sex (Table 4.40).

Table 4.40: Effect of Sex (Males & Females) on the Intercept, Concentration and/or Duration Parameter - AIC Comparison of Four Modeling Options

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1		Animal Species Route of Exposure Age	Animal Species Route of Exposure	120.1421
2	Sex	Animal Species Route of Exposure Age	Animal Species Route of Exposure	121.3965

3	Animal Species Route of Exposure Age Sex	Animal Species Route of Exposure	127.2218
4	Animal Species Route of Exposure Age	Animal Species Route of Exposure Sex	127.3384

All models use the two-level severity model with the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed.

The model will remain unstratified by sex. The concentration parameter will be stratified by animal species, route of exposure, and age and the duration parameter will be stratified by animal species and route of exposure. Table 4.41 presents the estimated parameters of the fitted exposure-response model, their standard errors and p-values.

Table 4.41: Parameter Estimates, Standard Errors, Z-test Statistics and P-values for Copper Excess Dietary Studies

Parameter	*Estimate*	*Std. Error*	*Z-test*	*P-value*
SEV1	-11.7940	3.5032	-3.3667	0.0008
INTERCEPT	0	0	NA	NA
HU:F:2:LG10CONC	7.1518	2.0216	3.5378	0.0004
HU:W:2:LG10CONC	21.4726	6.1598	3.4859	0.0005
MU:F:1:LG10CONC	19.5405	4.5539	4.2910	<0.0001
MU:F:2:LG10CONC	6.6814	1.5944	4.1901	<0.0001
MU:W:2:LG10CONC	2.6576	0.7304	3.6384	0.0003
RT:F:1:LG10CONC	3.6642	0.8842	4.1439	<0.0001
RT:F:2:LG10CONC	3.2087	0.7259	4.4205	<0.0001
RT:W:2:LG10CONC	2.3049	0.5312	4.3392	<0.0001
HU:F:LG10TIME	7.9845	1.9747	4.0435	<0.0001
HU:W:LG10TIME	12.5352	2.6000	4.8212	<0.0001
MU:F:LG10TIME	**-1.6628**	**1.0110**	**-1.6447**	**0.1000**
MU:W:LG10TIME	6.8095	2.0325	3.3503	0.0008
RT:F:LG10TIME	4.5487	1.4399	3.1591	0.0016
RT:W:LG10TIME	6.9169	2.3172	2.9851	0.0028

Two-level severity model uses the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) have been log transformed. Note. SEV, severity level; LG10, log transformed to the base 10; CONC, concentration coefficient; TIME, duration coefficient; HU, human; MU, mice; RT, rat; F, dietary study; W, drinking water study; 1, young animals (≤30 days of age); 2, mature animal (>30 days of age for rodents and >18 years for humans)..

The only estimate that is not statistically different from zero is the mice dietary stratum for the duration parameter. This is likely caused by the fact that there is very little variability across different durations of exposure for this stratum. In fact, there are only two

different durations of exposure. Figure 4.11 presents the ERC10-T100 for the stratified analysis.

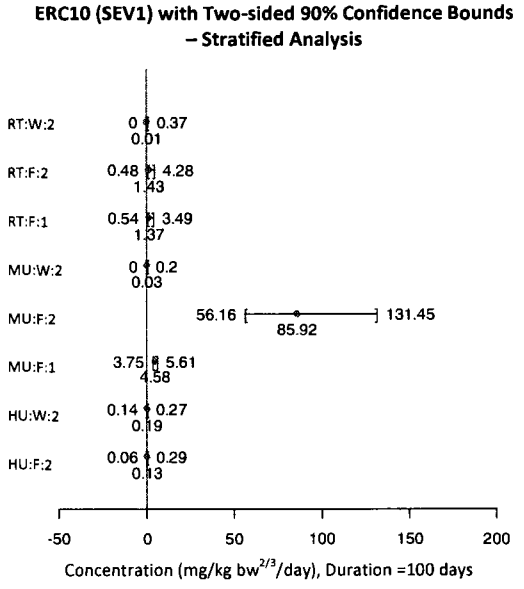


Figure 4.11: ERC10-T100 for the stratified analysis. Two-level severity model has been used with the logit link function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) transformed (log10). The concentration parameter is stratified by animal species, route of exposure and age and the duration parameter is stratified by animal species and route of exposure.

The confidence interval for the mice dietary stratum is much larger than the rat and human strata (Figure 4.11). The resulting ERC10-T100 for this stratum is also several magnitudes higher than the other ERC10-T100 estimates. This is due to the fact that the estimate for the mice dietary stratum had a negative coefficient for duration. Unlike the other strata, as duration increases the ERC10 line increases. As we are analyzing copper excess data we should expect that the ERC10 line would decrease with increasing duration of exposure. As there is minimal information on duration from the dietary mice studies it would be helpful to assume that the effect of duration was the same for rats and mice. It is important to recognize that the magnitude of the difference in parameter estimates may not be constant across all levels of each variable. There will likely always be a smaller difference between the parameter estimates for mice and rats compared to the parameter estimates for

humans. As there is a lack of variability across different durations of exposure among the mice studies, it would be ideal to have two parameter estimates for duration one for both mice and rats and another for humans. We could stratify the duration parameter by a new animal species variable with only two groups. Group 1 would include rats and mice and group 2 would include humans.

Table 4.42 presents the parameters in the exposure-response curve, the estimates, standard errors, z-test statistics and p-values for a model where the concentration parameter uses a three level variable and the duration parameter uses a two level variable for the animal species. All parameter estimates are now statistically different from zero and as expected all estimates for the duration parameter are positive.

Table 4.42: Parameter Estimates, Standard Errors, Z-test Statistics and P-values for Copper Excess Dietary Studies

Parameter	*Estimate*	*Std. Error*	*Z-test*	*P-value*
SEV1	-9.1517	2.4509	-3.7341	0.0002
INTERCEPT	0	0	NA	NA
HU:F:2:LG10CONC	7.5844	2.5167	3.0136	0.0026
HU:W:2:LG10CONC	20.9903	5.7992	3.6195	0.0003
MU:F:1:LG10CONC	7.4132	1.8487	4.0099	0.0001
MU:F:2:LG10CONC	2.3117	0.5985	3.8623	0.0001
MU:W:2:LG10CONC	2.1696	0.5231	4.1477	<0.0001
RT:F:1:LG10CONC	3.3123	0.6628	4.9974	<0.0001
RT:F:2:LG10CONC	3.0190	0.5657	5.3366	<0.0001
RT:W:2:LG10CONC	1.9608	0.5163	3.7982	0.0002
HU:F:LG10TIME	6.4854	1.4713	4.4079	<0.0001
HU:W:LG10TIME	10.9541	2.0842	5.2558	<0.0001
RM:F:LG10TIME	3.0002	0.9559	3.1386	0.0017
RM:W:LG10TIME	5.2827	1.4462	3.6528	0.0003

Two-level severity model uses the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) have been log transformed to the base 10. Note. SEV, severity level; LG10, log transformed to the base 10; CONC, concentration coefficient; TIME, duration coefficient; HU, human; MU, mice; RT, rat; F, dietary study; W, drinking water study; 1, young animals (≤30 days of age); 2, mature animal (>30 days of age for rodents and >18 years for humans).

To inform the concentration-duration relationship for humans (i.e. C^nT) the coefficient for concentration (β_1=7.58) is divided by the coefficient for duration (β_2=6.49). The resulting relationship is $C^{1.2}T$. In terms of whether the C-T metric ($\alpha + \beta 1 \log C + \beta 2 \log T$) can be reduced to $\alpha + \beta \log(CT)$, the Wald type chi-square test finds that the

estimate for the coefficient of concentration (β_1) is statistically different than the estimate for the coefficient of duration (β_2) ($p<0.0001$).

The previous section has determined the following: the simpler cumulative odds model is adequate to describe the copper excess data; the unrestricted cumulative model is too complex to consider all seven severity scores in the data; the unrestricted cumulative model with only three levels of severity is still too complex to consider different stratification options for the intercept, concentration and duration parameter; and finally, when the copper excess data is defined by a two-level severity model a series of model comparisons determined that the model best describes the variability in the database when the concentration parameter is stratified by animal species, route of exposure, and age and when the duration parameter is stratified by animal species and route of exposure.

4.6D Sensitivity Analyses

As in section 4.5, adding the acute exposure studies to the analysis increases the magnitude of the standard errors relative to the size of the parameter estimates. Parameter estimates for severity level 1 and several estimates for the duration parameter are no longer statistically significant (Table 4.43). Further background on acute exposure studies and their impact on the analysis will be discussed in Part 5.

Table 4.43: Parameter Estimates, Standard Errors, Z-test Statistics and P-values for Copper Excess Dietary Studies – Acute Exposure Studies Included in the Analysis

Parameter	*Estimate*	*Std. Error*	*Z-test*	*P-value*
SEV1	-0.00032	0.8572	-0.00038	0.9970
INTERCEPT	0	0	NA	NA
HU:F:2:LG10CONC	10.5418	4.5780	2.3027	0.0213
HU:W:2:LG10CONC	0.7383	0.3468	2.1289	0.0333
MU:F:1:LG10CONC	3.2785	1.0468	3.1320	0.0017
MU:F:2:LG10CONC	1.0095	0.2144	4.7087	<0.0001
MU:W:2:LG10CONC	0.8737	0.2656	3.2897	0.0010
RT:F:1:LG10CONC	1.7560	0.3652	4.8089	<0.0001
RT:F:2:LG10CONC	1.6411	0.3595	4.5649	<0.0001
RT:W:2:LG10CONC	0.8243	0.2059	4.0039	0.0001
HU:F:LG10TIME	1.6012	0.9786	1.6363	0.1018
HU:W:LG10TIME	0.2435	0.4005	0.6080	0.5432
RM:F:LG10TIME	-1.0142	0.5338	-1.9000	0.0574
RM:W:LG10TIME	-0.0998	0.5561	-0.1795	0.8575

Two-level severity model uses the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) have been log transformed to the base 10. Note. SEV, severity level; LG10, log transformed to the base 10; CONC, concentration coefficient; TIME, duration coefficient; HU, human; MU, mice; RT, rat; F, dietary study; W, drinking water study; 1, young animals (≤30 days of age); 2, mature animal (>30 days of age for rodents and >18 years for humans).

In section 4.5, human drinking water studies were filtered from the analysis to look at how these studies impact the ERC10 estimates for humans. When copper is administered in drinking water in subacute and subchronic exposure studies, responses that are observed (e.g. gastro intestinal symptoms) may not be a consequence of long-term elevated copper intake but rather acute responses to the repeated exposures. Similar to what was seen with the cumulative odds model in section 4.5, the exclusion of the drinking water studies on humans from the analysis does not appear to have an effect on the ERC10-T100 for the human dietary stratum (Table 4.44). These observations will be retained in the analysis.

Table 4.44: ERC10-T100 and Two-sided 90% Confidence Intervals (CI) for the Human Dietary Stratum – With and Without Human Drinking Water Studies

Model	*ERC10-T100 (90% CI)*
With human drinking water studies*	0.16 (0.07, 0.36)
Without human drinking water studies	0.16 (0.07, 0.37)

*Studies include: Araya 2004, Araya 2003c, and Pizarro 1999b
Two-level severity model uses the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) have been log transformed to the base 10. Concentration was stratified by the animal species, route of exposure and age and duration was stratified by the animal species and route of exposure.

In section 4.5B a sensitivity analysis was conducted with the cumulative odds model to look at the impact of one rat study by Liu and associates that used a less soluble form of copper (Liu et al., 1986). Table 4.45 presents the estimated ERC10 values for an exposure duration of 100 days for the rat dietary stratum, with and without this study. Consistent with the results in section 4.5, there is a minimal change (less than 12%) in the value of this estimate.

Table 4.45: ERC10-T100 and Two-sided 90% Confidence Intervals (CI) for the Rat Dietary Stratum – Effect of Solubility

Model	*ERC10 (90% CI)*
With Liu et al., (1986)	2.0703 (0.7442, 5.7595)
Without Liu et al., (1986)	2.3562 (0.7723, 7.1882)

Two level severity model with the link function was used to generate the ERC10-T100 estimates for weanling rats. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) parameters were log transformed. Concentration was stratified by the animal species, route of exposure, and age and duration was stratified by the animal species and route of exposure.

4.6E Data Review for Outliners

CatReg was used to generate plots (Figure 4.12 and Figure 4.13) of the generalized deviance residuals versus the observation number. Between Figure 4.12 and Figure 4.13 all observations for the six strata are plotted. There does not appear to be any particular stratum that is poorly described by the exposure-response curve.

The observation with the highest deviance residual is associated with the human dietary stratum, and is from a study by Turnlund et al. (2004). This is an important study as it measured a broad range of copper indices including immune cell functioning, antioxidant status and other traditional indicators of copper status (e.g., serum copper and ceruloplasmin) not found in other studies.

Deviance Plot of the Observations on Human and Mice Dietary and Water Studies in the Two-Level Severity Model – Copper Excess Data

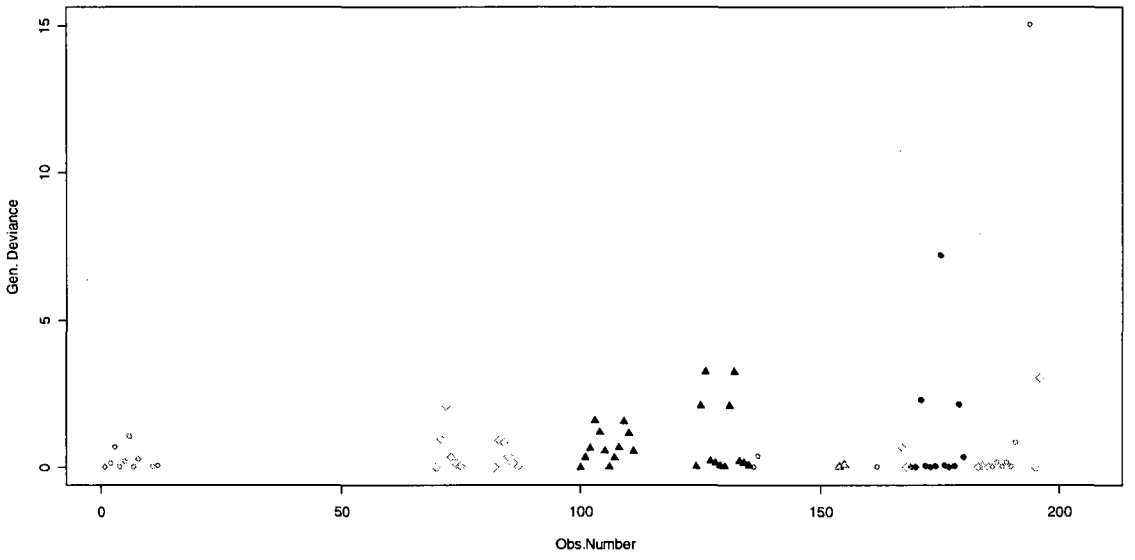


Figure 4.12: Deviance plot by observation #. Human dietary studies = ○, human water studies =●, weanling mice dietary studies =◇, mature mice dietary studies =△, mature mice drinking water studies =▲. Note. Gen., general; Obs, observation.

Deviance Plot of the Observations on Rat and Human Dietary and Water Studies in the Two-Level Severity Model – Copper Excess Data

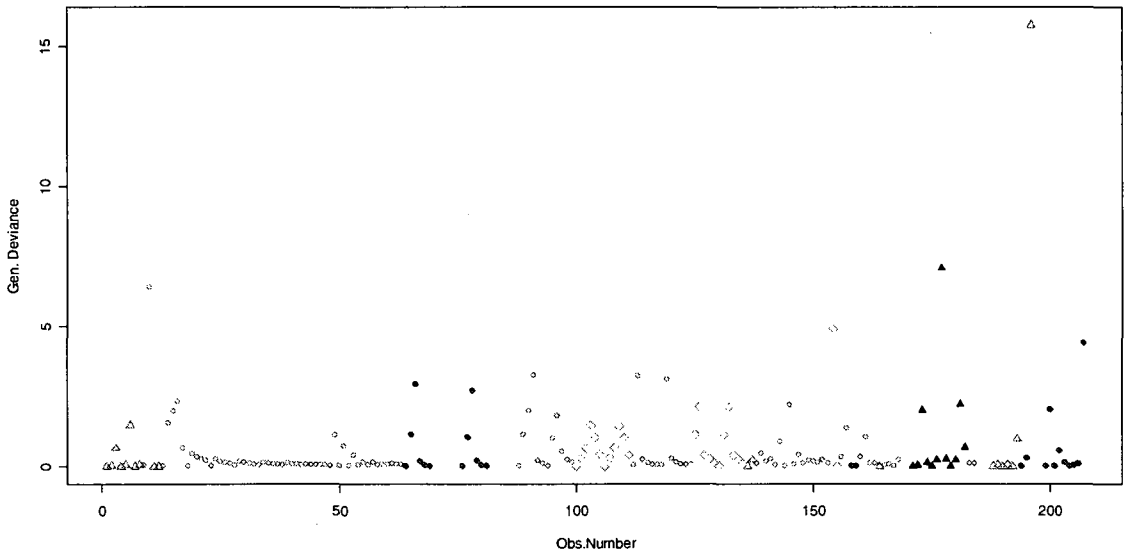


Figure 4.13: Deviance Plot by Observation #. Weanling rat dietary studies = ○, mature rat dietary studies =●, mature rat drinking water studies =△, adult human dietary studies =▲, adult human drinking water studies =◇. Note. Gen., general; Obs, observation.

4.6F Model Fit

The two-level severity model defined in sections 4.6A to 4.6C was used to generate a series of plots for each stratum including a plot of the ERC10 line for severity level 1 or

greater with two-sided 90% confidence intervals and a plot of all the ERC10 lines for each severity level. Just to note, when the ERC10 lines are defined at severity level 1, this would have represented severity level 2 or greater in the original severity scoring scheme. In this categorical regression analysis of the copper excess data, the acceptable range of oral intake (AROI) was thought of as being defined by the ERC10 at severity level 2 or greater. Plots for the human strata are presented below and plots for rats and mice can be found in Appendix I. As emphasized in section 4.5 with the cumulative odds model the purpose of these figures is to: look at the impact of duration in the exposure-response model; qualitatively assess model fit by determining whether the ERC10 lines for each severity level are below their corresponding observations; and determine the extent of variability in observed toxicity (i.e. overlap in the assignment of severity scores).

Figures 4.14 and 4.15 present a plot of the ERC10 line for severity level 2 or greater with 90% two-sided confidence intervals for the human dietary studies and human drinking water studies, respectively. As expected, in each figure the ERC10 line has a negative slope as longer durations require a lower concentration to achieve the estimated 10% response probability.

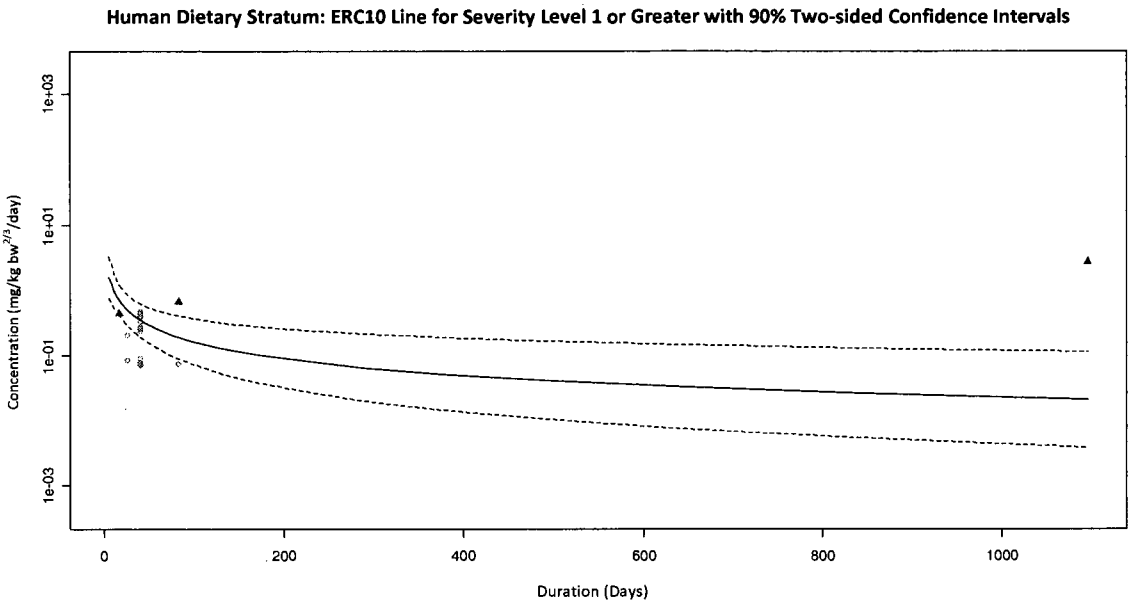


Figure 4.14: ◌ = severity level 0, ▲ = severity level 1. Two-level severity model uses the logit link function. Concentration and duration log transformed. The concentration parameter is stratified by animal species, route of exposure and age and the duration parameter is stratified by the animal species and route of exposure.

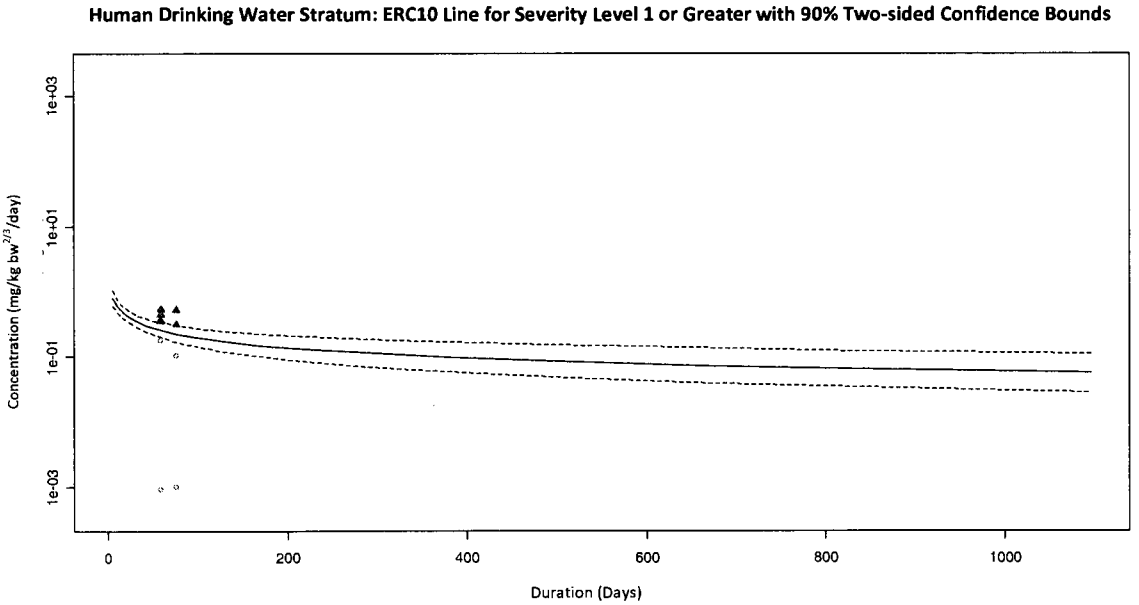


Figure 4.15: ◌ = severity level 0, ▲ = severity level 1. Two-level severity model uses the logit link function. Concentration and duration log transformed. The concentration parameter is stratified by animal species, route of exposure and age and the duration parameter is stratified by the animal species and route of exposure.

In the cumulative odds model, all ERC10 lines fell below their corresponding observations. In the two-level severity model, the ERC10 line falls below all corresponding

observations in all strata except for the human dietary stratum. While the ERC10 line for the human dietary stratum falls below its corresponding observations at subchronic exposures, it does not fall below its corresponding observations at subacute exposures. There are, however, very few observations in this stratum. The cumulative odds model which uses several levels of severity appears to fit the human dietary data better than the two-level severity model.

There is some overlap in the assignment of severity scores within the subacute exposure range in the mature rat dietary stratum, weanling rat dietary stratum and the mature rat drinking water stratum. There is very little overlap in the assignment of severity scores in the strata for mice and humans. Overall the two-level severity model of the copper excess data appears to fit the data well.

Figure 4.16 plots all the ERC10 lines by concentration and duration for each stratum. It is difficult to visualize which ERC10 lines correspond do which strata; however, there are no options in CatReg that will improve the presentation of the plotting options available. At T=800 days, the ordering of the curves by their corresponding strata from top to bottom are: weanling mice dietary stratum, weanling rat dietary stratum, mature rat dietary stratum, mature mice dietary stratum, human drinking water stratum, human dietary stratum, mice drinking water stratum and the rat drinking water stratum. When comparing the ERC10 lines for different strata one should focus on the range of exposures where data is available. There is very little difference in the slopes of the ERC10 lines for the human dietary and drinking water strata. The slope of ERC10 curve for the rat drinking water stratum is much greater than the rat dietary stratum. The same is true for the mice strata. For rats and mice, it appears as though the rate at which concentration and duration impact severity of response is much greater when copper is administered in drinking water. The is very little

difference in the slopes of the ERC10 lines for age among rat studies; however, there is a much greater difference among mature and young mice. This figure demonstrates that the animal species, route of exposure and age will impact how the exposure-response curve for copper excess is characterized.

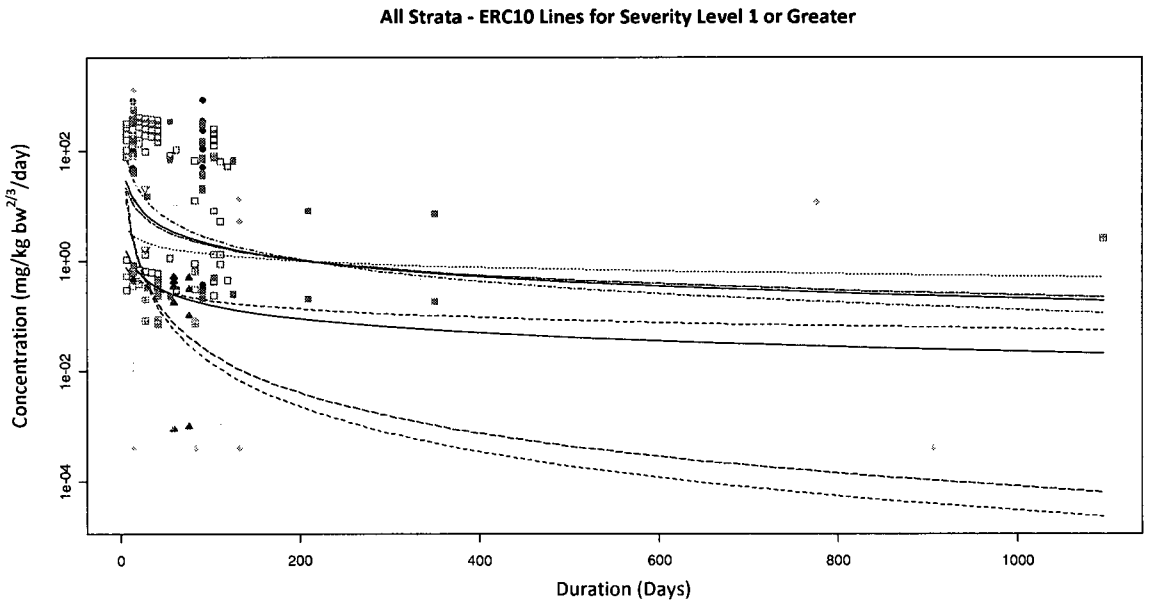


Figure 4.16: Two-level severity model with logit function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed. Concentration parameter is stratified by animal species, route of exposure, and age and the duration parameter is stratified by animal species and route of exposure. ⬚ = human dietary stratum, ▲ = human drinking water stratum, ▽ = weanling mice dietary stratum, ● = mature mice dietary stratum, ◌ = mature mice drinking stratum, □ = weanling rat dietary stratum, ▩ = mature rat dietary stratum, = mature rat drinking water stratum.

4.6G Final Estimates

Table 4.46 presents the ERC10-T100 for severity level 1 or greater for each stratum in the final model. After accounting for interspecies differences due to an approximate of surface area, rats require a 12.9 times greater concentration of copper in diet and mice require 16.1 times greater concentration of copper in diet to produce the same level of severity as humans at T=100 days of exposure. After accounting for interspecies differences using surface area as an approximate means of scaling between species, mice require a 1.3 times greater concentration to produce the same level of severity as rats. The difference in the ERC10 estimates between animals and humans is much less in the two-level

severity model compared to the cumulative odds model. This will be discussed further in Part 5. Comparisons of the final estimates and model selection options between the cumulative odds model and the two-level severity model for the copper excess data will presented in section 4.10.

The ERC10-T100 estimate for human drinking water studies at severity level 2 or greater is 0.1939 mg/kg $bw^{2/3}$/day. If we assume that the background diet provides 0.07 mg/kg $bw^{2/3}$/day (Baker 1999a) and we add this to the ERC10 estimate for human drinking water studies, the resulting value is 0.2639 mg/kg $bw^{2/3}$/day. Unlike the cumulative odds model that found very little difference between the ERC10-T100 for the human drinking water stratum and the human dietary stratum after adding a habitual intake of copper from diet, the two-level severity model results in a large difference between these two strata.

Table 4.46: ERC10-T100 with Two-sided 90% Confidence Intervals (CI) for Severity Level 1 or Greater

Data Included in the Analysis	*ERC10 (90% CI)*
Humans Dietary Studies	0.1610 (0.0718, 0.3611)
Humans Water Studies	0.1939 (0.1398, 0.2690)
Rats Mature Dietary Studies	2.0703 (0.7442, 5.7595)
Rats Young Dietary Studies	1.9411 (0.7863, 4.7918)
Rats Mature Water Studies	0.0144 (0.0010, 0.2097)
Mice Mature Dietary Studies	2.5865 (0.7339, 9.1159)
Mice Young Dietary Studies	1.3449 (0.9052, 1.9983)
Mice Mature Water Studies	0.0217 (0.0032, 0.1466)

Two-level severity model uses the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed. Concentration is stratified by the animal species, route of exposure, and age and duration is stratified by the animal species and route of exposure.

Table 4.47 presents the ERC10 estimates for severity level 1 or greater for each stratum at T=25, 50 and 100 days. The impact of duration appears to be the most significant for mice where approximately 2.5 times greater concentration is required to produce the same level of severity as duration decreases from 100 days to 50 days and from 50 days to 25 days. For humans, approximately 1.8 times greater concentration is required to

produce the same level of severity as duration decreases from 100 to 50 days and from 50 to 25 days.

Table 4.47: ERC10 Estimates for Severity Level 1 or Greater with 90% Two-sided Confidence Intervals (CI) at T=25, 50 and 100 days of Exposure

Data Included in the Analysis	*ERC10 (90% CI)*		
	T=25	*T=50*	*T=100*
Humans Dietary Studies	0.5267 (0.3092, 0.8974)	0.2912 (0.1566, 0.5416)	0.1610 (0.0718, 0.3611)
Humans Water Studies	0.3997 (0.3265, 0.4895)	0.2784 (0.2177, 0.3560)	0.1939 (0.1398, 0.2690)
Rats Mature Dietary Studies	8.2102 (2.7026, 24.9413)	4.1228 (1.4724, 11.5442)	2.0703 (0.7442, 5.7595)
Rats Young Dietary Studies	6.8134 (2.7296, 17.0073)	3.6367 (1.5160, 8.7236)	1.9411 (0.7863, 4.7918)
Rats Mature Water Studies	0.6033 (0.1183, 3.0757)	0.0932 (0.0121, 0.7202)	0.0144 (0.0010, 0.2097)
Mice Mature Dietary Studies	15.6351 (4.9346, 49.5395)	6.3593 (1.9864, 20.3584)	2.5865 (0.7339, 9.1159)
Mice Young Dietary Studies	2.3570 (1.5835, 3.5083)	1.7805 (1.2186, 2.6014)	1.3449 (0.9052, 1.9983)
Mice Mature Water Studies	0.6334 (0.1488, 2.6961)	0.1771 (0.0231, 0.5942)	0.0217 (0.0032, 0.1466)

Two level severity model with the logit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed. Concentration is stratified by the animal species, route of exposure, and age and duration is stratified by the animal species and route of exposure. Note. T, duration of exposure (days).

In this section we learned that there was insufficient data in the copper toxicity database and there was too much variation in the number of severity scores and variables of interest (e.g. animal species, route of exposure, age and sex) to use the unrestricted cumulative model. A two-level severity model appears to fit the copper excess data well as indicated in section 4.6F. Using a two-level severity model (section 4.6) versus a six level severity model (section 4.5) appears to greatly impact the final form of the model in terms of which parameters are stratified, the magnitude of difference between each strata and the resulting ERC10 estimates. After the copper deficiency data is modeled, section 4.10 will provide a detailed comparison of all the models used in the analysis.

SECTION 4.7 COPPER DEFICIENCY MODEL SELECTION – EXPOSURE METRIC AND LINK FUNCTION

Section 4.4 compared a series of AIC values for different link functions, transformation options and dose metrics for the copper excess data. This section will do the same for the copper deficiency data using the cumulative odds model. The pig studies were omitted from the analysis due to their scarcity in the database. In Table 4.48 the AIC is listed for the 12 different modeling options defined by three different link functions (logit, probit, and C log-log); two transformation options (log and linear) and 5 different dose metrics (mg/day, mg/kg bw/day, mg/kg $bw^{1/4}$/day, mg/kg $bw^{2/3}$/day, and mg/kg $bw^{3/4}$/day).

Table 4.48: AIC for 12 Modeling Options for Copper Deficiency

Link function	*C*	*T*	AIC for Each Dose Metric				
			Mg/d	*Mg/kg bw/d*	*Mg/kg $bw^{1/4}$/d*	*Mg/kg $bw^{2/3}$/d*	*Mg/kg $bw^{3/4}$/d*
Logit	Linear	Linear	711.0419	514.3146	589.6303	434.1759	478.7236
Logit	Linear	Log	712.2040	511.1258	592.3315	434.7260	478.5426
Logit	Log	Linear	712.2088	514.3866	591.2118	434.1967	478.7395
Logit	Log	Log	713.7829	511.2093	592.3558	434.7609	478.5601
Probit	Linear	Linear	708.5150	518.7908	599.6931	432.1488	481.4176
Probit	Linear	Log	709.4185	517.7919	599.5356	434.4672	482.9107
Probit	Log	Linear	708.4595	518.8718	602.618	432.2104	481.4296
Probit	Log	Log	709.3174	517.8761	602.7241	434.4989	482.9237
C Log-log	Linear	Linear	715.7388	514.5548	570.4145	422.185	470.7226
C Log-log	Linear	Log	715.9776	505.7347	572.2651	417.4139	464.6375
C Log-log	Log	Linear	724.8997	514.6525	572.2661	422.1971	470.7417
C Log-log	Log	Log	726.5249	505.8695	573.4502	417.4278	464.6946

Cumulative odds model is used in the comparisons. Note. C, concentration; T, duration of exposure; C Log-Log, complementry log-log.

The lowest values for the AIC are associated with the models where concentration was defined in mg/kg $bw^{2/3}$/day. The complementry log-log link function produces the lowest AIC value; however, this link function tends to be easier to overparameterize than the other link functions. This means that it cannot handle as many variables because of the simplicity of the log-log transformation and the linear relationship that results from the use of this transformation. When the model is further stratified using this link function, CatReg presents several error messages in the calculation of the model parameters. The program

recommends the use of the logit or probit link function when the complementry log-log link function produces these errors messages; consequently, the probit link function will be used for the copper deficiency models. There are negligible differences among the different transformation options. As the concentration and duration parameters in the copper excess model were both transformed (log10), the log of concentration and duration will be selected for the copper deficiency model. All analyses in section 4.8 and 4.9 will take the log10 of concentration in mg/kg $bw^{2/3}$/day and the log10 of duration in days, and will use the probit link function.

4.8 CUMULATIVE ODDS MODEL – COPPER DEFICIENCY

In the following subsection (4.8A) a series of stratification options were compared in order to select a model that is sufficiently accurate by considering different parameters (i.e. animal species, age, and sex) and stratification options (i.e. stratification of the intercept, concentration and/or duration parameter) but achieves this aim as simply as possible.

4.8A Model Selection – Stratification Options

There are currently five levels of severity (severity level 0 to 4) among the copper deficiency data. As discussed in the descriptive analysis, severity level 4 to 6 had to be combined as there were no observations at severity level 5 and only 1 observation at severity level 6. The copper deficiency data was fit with the cumulative odds model with no stratification options.

In the cumulative odds model, intercept parameters are estimated for each level of severity. Table 4.49 presents the results from a Wald-type chi-square test of the null hypothesis that the estimates for neighboring severity scores are equal. The p-values for all combinations are significant, indicating that there is evidence that the parameters are not equal. The analysis will proceed using all five levels of severity (0-4).

Table 4.49: Equality Test for Neighboring Severity Coefficients – Cumulative Odds Model* of the Copper Deficiency Data

Test of Equality	*Chi-square*	*df*	*P-value*
SEV1 & SEV2	10.9797	1	<0.0001
SEV2 & SEV3	27.5371	1	<0.0001
SEV3 & SEV4	52.7894	1	<0.0001

*Cumulative odds model uses the probit function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) transformed (log10).

The AIC from a series of models were compared to look at the impact of the animal species on the intercept, concentration and duration parameters (Table 4.50).

Table 4.50 Effect of the Animal Species on the Intercept, Concentration and/or Duration Parameter - AIC Comparison of Eight Modeling Options

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1	--	--	--	486.3394
2	Animal Species			468.8181
3	--	Animal Species	--	471.6261
4	--	--	Animal Species	469.7435
5	Animal Species	Animal Species		469.607
6	Animal Species		Animal Species	465.4751
7		Animal Species	Animal Species	471.6806
8	Animal Species	Animal Species	Animal Species	464.5289

Cumulative odds model is used with the probit function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) transformed (log10).

While model 8 is associated with the lowest AIC, the animal species does not have a significant effect on the intercept, concentration or duration parameter (Table 4.51). The stratification options in model 6, which has the second lowest AIC, are not statistically significant (Table 4.51). Model 2, where the intercept is stratified by the animal species is associated with the third lowest AIC. In this model, the animal species does have a significant effect on the intercept parameter (Table 4.52). The difference between the deviances of model 2 and model 1 (unstratified model) were tested against a chi-square

distribution with 2 degrees of freedom ($df_{model2} = 4$, $df_{model1} = 2$). The difference between the deviances of the two models (475.9259-468.2044=7.7215) is significant (p=0.0211, df = 2). In further analyses the intercept will be stratified by the animal species.

Table 4.51: Test for the Effect of Animal Species in Models 8, 6 and 2

Model # and Stratification Options	*Chi-square*	*df*	*P-value*
Model 8			
Intercept Stratified by Animal Species	5.3551	2	0.06873
Concentration Stratified by Animal Species	2.8419	2	0.2415
Duration Stratified by Animal Species	3.8978	2	0.1424
Model 6			
Intercept Stratified by Animal Species	4.1663	2	0.1245
Duration Stratified by Animal Species	4.5309	2	0.1038
Model 2			
Intercept Stratified by Animal Species	34.0258	2	<0.0001

Cumulative odds model is used with the probit function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) transformed (log10).

Table 4.52 presents a series of models where the intercept, concentration and/or duration parameter is stratified by age. In every model, the intercept is stratified by the animal species.

Table 4.52: Effect of Age on the Intercept, Concentration and/or Duration Parameter - AIC Comparison of Eight Modeling Options

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1	Animal Species	--	--	468.8181
2	Animal Species Age	--	--	458.5375
3	Animal Species	Age		456.2858
4	Animal Species		Age	460.983
5	Animal Species Age	Age		459.4455
6	Animal Species Age		Age	454.5243
7	Animal Species	Age	Age	458.2194
8	Animal Species Age	Age	Age	456.2113

Cumulative odds model is used with the probit function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) transformed (log10).

Model 6, where the intercept and duration parameters are stratified by age is associated with the lowest AIC; however, the effect of age on the intercept and duration parameter is not significant (Table 4.53). In model 3, the effect of age on the concentration parameter is significant (Table 4.53). The difference between the deviances of model 3 and model 1 (unstratified by age) was tested against a chi-square distribution with 1 degree of freedom ($df_{model3} = 5$, $df_{model1} = 4$). The difference between the deviances of the two models (454.9351-439.1236=15.8115) is significant (p=0.0001, df = 1). In further analyses the intercept will be stratified by the animal species and the concentration parameter will be stratified by age.

Table 4.53: Test for Effect of Age on the Intercept and Duration Parameter

Model # and Stratification Options	*Chi-square*	*df*	*P-value*
Model 6			
Intercept Stratified by Age[a]	5.6806	2	0.0584
Duration Stratified by Age	3.0786	1	0.0793
Intercept Stratified by Animal Species[b]	9.1648	3	0.0272
Model 3			
Concentration Stratified by Age	8.4344	1	0.0037
Intercept Stratified by Animal Species	11.5525	2	0.0031

Cumulative odds model is used with the probit function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) transformed (log10).
[a]Controlling for the animal species
[b]Controlling for age

To examine the effect of sex, this variable was categorized into three categories: 'males', 'females' and 'both'. Table 4.54 presents the AIC for four models where the intercept, concentration and/or duration parameter was stratified by sex. The AIC does not decrease when the intercept, concentration or duration parameters are stratified by sex (Table 4.54).

Table 4.54: Effect of Sex (Both, Males & Females) on the Intercept, Concentration and Duration Parameter - AIC Comparison of Four Modeling Options

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1	Animal Species	Age	--	456.2858
2	Animal Species Sex	Age	--	457.1427
3	Animal Species	Age Sex	--	461.3208
4	Animal Species	Age	Sex	457.9918

Cumulative odds model is used with the probit function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) transformed (log10).

Another approach to looking at the effect of sex in the model is to remove all the studies that did not report their findings separately for males and females. After removing all the studies that did not report their findings separately for males and females, the number of observations decreases from 208 to 192. Using a two-level sex variable (i.e. males and females) the AIC does not decrease when the intercept, concentration or duration parameters are stratified by sex (Table 4.55).

Table 4.55: Effect of Sex (Males and Females) on the Intercept, Concentration and Duration Parameter - AIC Comparison of Four Modeling Options

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1	Animal Species	Age	--	434.7977
2	Animal Species Sex	Age	--	435.1599
3	Animal Species	Age Sex	--	438.5213
4	Animal Species	Age	Sex	436.625

Cumulative odds model is used with the probit function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) transformed (log10).

Section 4.2 identified several studies on copper deficiency in the database that use copper carbonate, a less soluble form of copper. Essentially, using a less soluble form of copper would result in a more severe state of copper deficiency, as less copper would be

absorbed in the gastrointestinal tract. Table 4.56 presents the AIC for four models where the intercept, concentration and/or duration parameter was stratified by solubility (low solubility versus high solubility). In each model the intercept is also stratified by the animal species and the concentration parameter by age. Model 2, where the intercept is stratified by the animal species has a slightly lower AIC compared to model 1 (unstratified by solubility). The effect of solubility on the intercept after controlling for the animal species is marginally significant (p=0.0528, df =2) and the difference between the deviances of the two models (439.1236-430.5122=8.6114) is significant (p=0.0135, df = 2).

Table 4.56: Effect of Solubility on the Intercept, Concentration and Duration Parameter - AIC Comparison for Four Models

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1	Animal Species	Age	--	456.2858
2	Animal Species Solubility	Age	--	452.5122
3	Animal Species	Age Solubility	--	459.9956
4	Animal Species	Age	Solubility	459.1086

Cumulative odds model is used with the probit function. Concentration (mg/kg $bw^{2/3}$/day and duration (days) transformed (log10).

As mentioned earlier, if the studies using less soluble forms of copper had an impact on the analytical results, we would expect that excluding these observations would decrease the value of the ERC10, since a less soluble form of copper would results in a more severe state of copper deficiency. Table 4.57 presents the ERC10 estimates for two modeling options. In model 1 the intercept is stratified by the animal species and solubility (low solubility versus high solubility) and concentration is stratified by age. In model 2 the intercept is only stratified by the animal species and the concentration parameter is stratified by age. For humans and rats there is minimal difference (0.02%) in the ERC10-T100 estimates or the width of the confidence intervals between the two models. For mice, the

ERC10-T100 is lower for the high solubility stratum than the low solubility stratum. This trend is not found in the weanling and mature rat strata.

Table 4.57: ERC10-T100 and Two-sided 90% Confidence Intervals (CI) for Weanling and Mature Rats and Mice – Effect of Solubility

Strata in Model 1[a]	ERC10-T100, (90% CI)	*Strata in Model 2*[b]	ERC10-T100, (90% CI)
HU:2	0.1620 (0.1118, 0.2348)	HU:2	0.1647 ((0.1137, 0.2385)
MU:S:2	0.3023 (0.1557, 0.5869)	MU:2	0.4507 (0.2453, 0.8277)
MU:U:2	0.6820 (0.2956, 1.5734)		
MU:S:1	0.3839 (0.2159, 0.6825)	MU:1	0.524 (0.3159, 0.8694)
RT:S:1	0.348 (0.2506, 0.4834)	RT:1	0.3448 (0.2514, 0.4727)
RT:U:1	0.2548 (0.1885, 0.3444)		
RT:S:2	0.2674 (0.1767, 0.4049)	RT:2	0.2689 (0.1800, 0.4017)
RT:U:2	0.1811 (0.1242, 0.2642)		

[a]In model 1 the intercept is stratified by solubility and animal species; concentration is stratified by age.
[b]In model 2 the intercept is stratified only by animal species; concentration is stratified by age
Cumulative odds model with the probit link function. Concentration (mg/kgbw$^{2/3}$/day and duration (days) transformed (log10). Note. MU, mice; RT, rat; S, more soluble form of copper; U, less soluble form of copper; 2, mature animals (>30 days of age) or adult humans (≥18 years of age); 1 = young animals (≤30 days of age).

In Figure 4.17, data from the adult mice dietary stratum is plotted with a model that is not stratified by solubility. It is important to note that observations associated with severity level 4 are above observations at severity levels 2 and 3. The ERC10 line for severity level 4 or greater is not above its corresponding observations. In Figure 4.18, data from the adult mice dietary stratum for low soluble forms of copper are plotted with a model that has been stratified by solubility. One can see that the data points corresponding to severity level 4 belong to studies where low soluble forms of copper have been used. The ERC10 line for severity level 4 is now above one of the two observations at severity level 4. In Figure 4.19, this same model is used to plot the data for the adult mice stratum for more soluble forms of copper. It is important to note that at this time there are no observations assigned a severity level 4 in this stratum. The large difference between the estimates for low and high solubility in the mature mice stratum may be due to the fact that studies using more soluble forms of copper have not yet used a low enough dose that would lead to responses associated with a

severity level 4 or greater. Due to the lack of data among mice, and the minimal impact of solubility among the rat stratum, the more simplified model will be used.

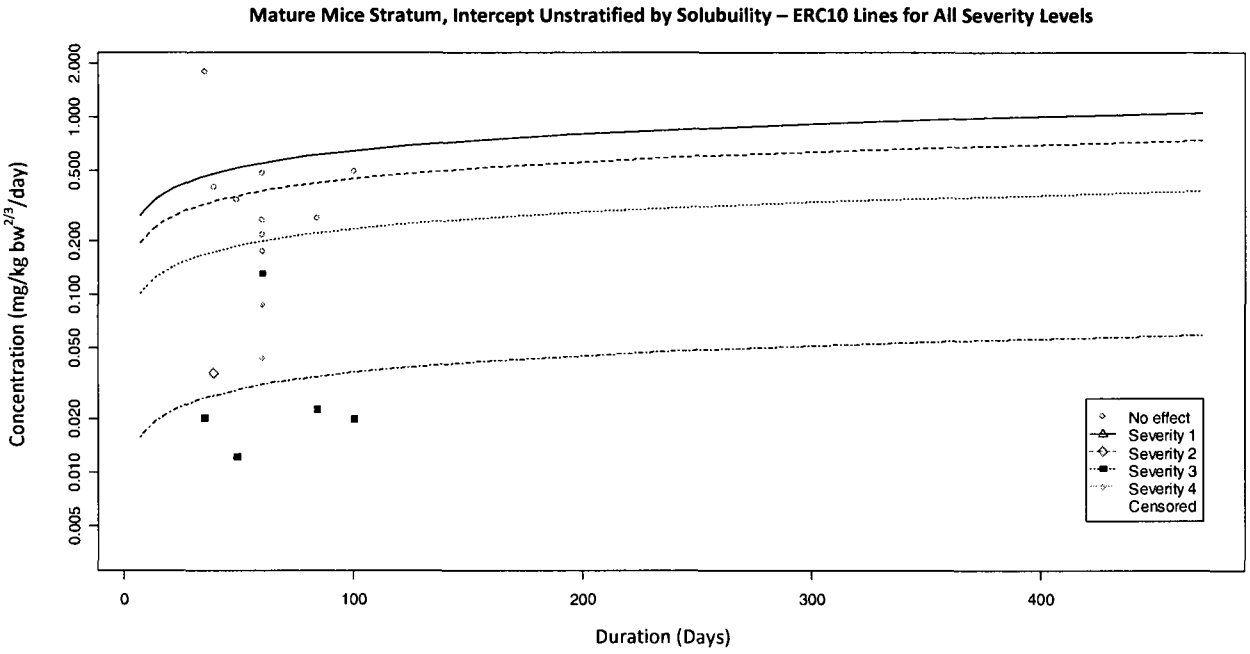


Figure 4.17: Cumulative odds model uses the probit link function. Concentration (mg/kg bw$^{2/3}$/day) and duration (days) are log transformed. Intercept parameter is stratified by animal species and the concentration parameter is stratified by age.

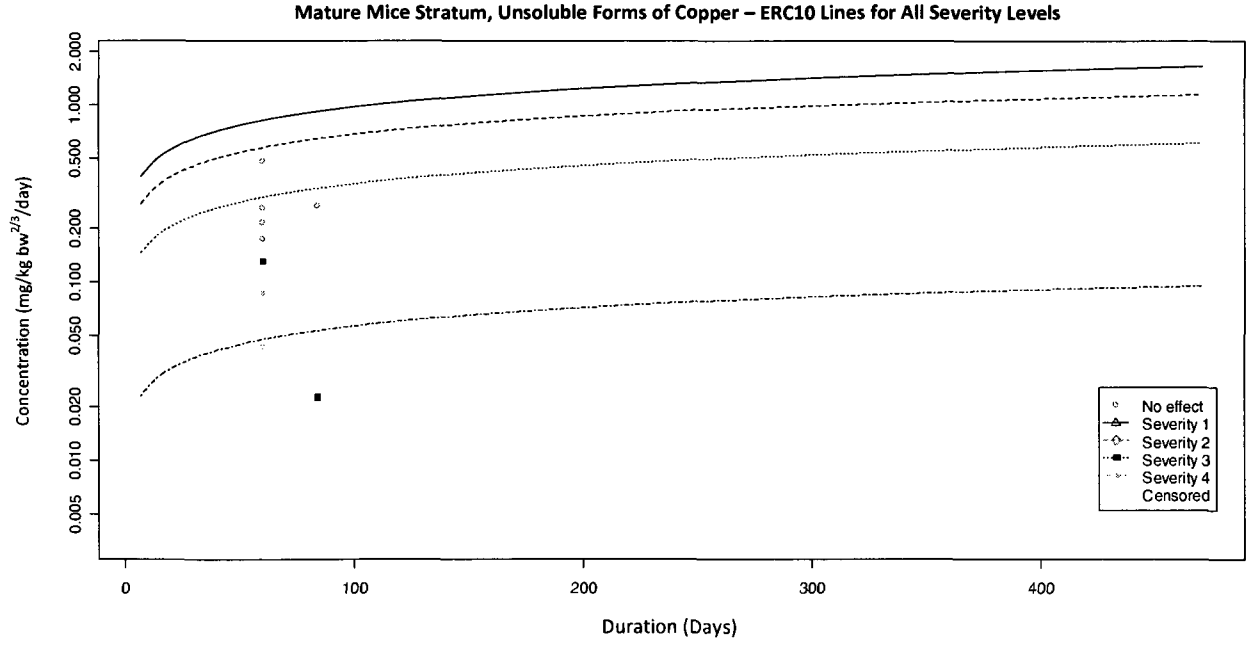


Figure 4.18: Cumulative odds model uses the probit link function. Concentration (mg/kg bw$^{2/3}$/day) and duration (days) are log transformed. Intercept parameter is stratified by animal species and solubility; concentration parameter is stratified by age.

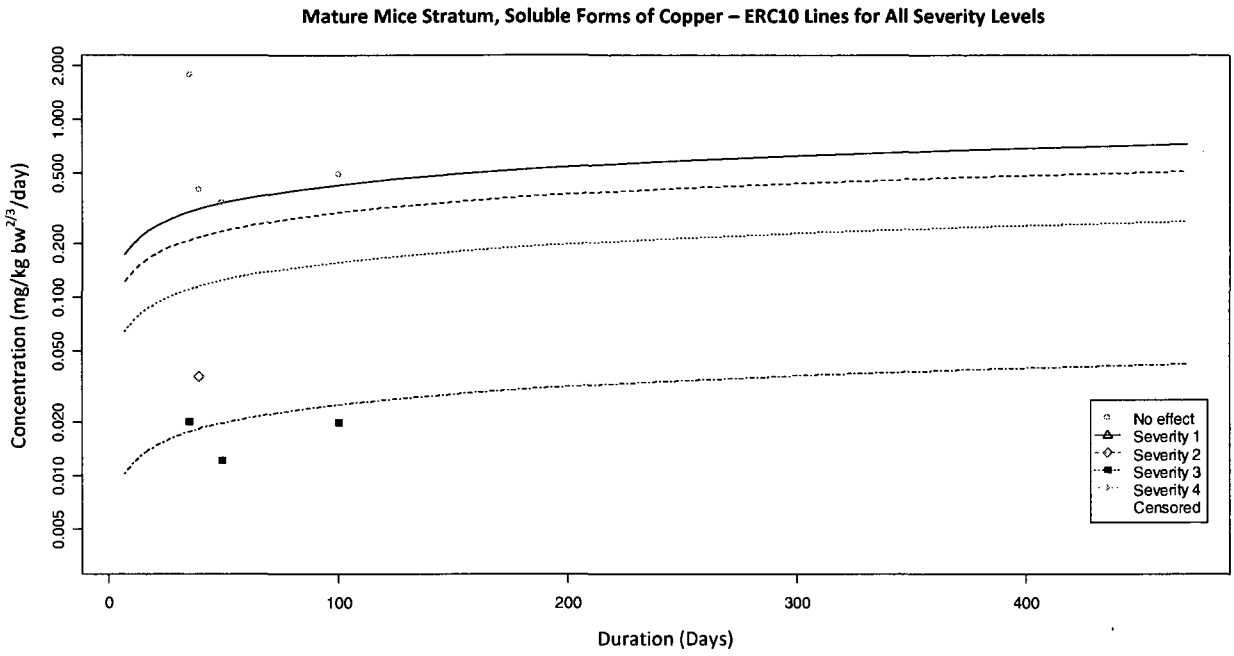


Figure 4.19: Cumulative odds model uses the probit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed. Intercept parameter is stratified by animal species and the concentration parameter is stratified by age.

The final model uses the probit link function, transforms concentration and duration (log 10), and stratifies the intercept by animal species and the concentration parameter by age. Table 4.58 presents the parameter estimates in the final model including their standard errors and p-values. While duration is not significant in the final model, it will be retained in the analysis for the series of figures in section 4.8B which plot the ERC10 line by concentration and duration. Removing duration from the exposure-response model collapses all the data together at one time point which reduces the ability to evaluate the degree of overlap in the assignment of severity scores. Furthermore, CatReg will not plot ERC10 lines when there is no variability in the duration of exposure.

Table 4.58: Parameter Estimates, Standard Errors, Z-test Statistics and P-values for the Cumulative Odds Model* of the Copper Deficiency Data

Parameter	*Estimate*	*Std. Error*	*Z-test*	*P-value*
SEV1	-4.6444	0.9010	-5.1547	<0.0001
SEV2	-5.0618	0.9051	-5.5925	<0.0001
SEV3	-5.8226	0.9185	-6.3391	<0.0001
SEV4	-7.9752	0.9994	-7.9797	<0.0001
HU:INTERCEPT	0.0000	0.0000	NA	NA
MU:INTERCEPT	1.1625	0.3684	3.1553	0.0016
RT:INTERCEPT	0.5710	0.2662	2.1452	0.0319
1:LG10CONC	-3.2905	0.3084	-10.6696	<0.0001
2:LG10CONC	-2.6720	0.2407	-11.0997	<0.0001
LG10TIME	0.8456	0.4936	1.7130	0.0867

* Cumulative odds model uses the probit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) log transformed (log10). Note. SEV, severity level; LG10, log transformed to the base 10; CONC, concentration coefficient; TIME, duration coefficient; HU, humans; MU, mice; RT, rats; 2, mature animals (>30 days of age) or adult humans (≥18 years of age); 1 = young animals (≤30 days of age).

Figures 4.20a-d present the results of the pooled and stratified analyses for severity level 1, severity level 2, severity level 3, and severity level 4. Comparing the ERC10-T100 estimates in the stratified analysis can help determine whether there are practical differences between the ERC10 estimates. The vertical line in each figure represents the pooled ERC10 with 90% two-sided confidence intervals for T=100 days of exposure. The horizontal lines are the stratum-specific ERC10-T100 estimates. There are large differences between the human estimates and the estimates for rats and mice. However, the difference is not as pronounced as in the analyses of the copper excess data. This will be discussed further in Part 5. The ERC10 for humans falls outside the pooled confidence interval for severity levels 1 to 3. For severity levels 1 to 4 the human estimates in the stratified analysis are more similar to the estimates in the pooled analysis compared to the estimates for rats and mice. For severity levels 1-4 the width of the confidence intervals for the pooled analysis are similar to or less than the width of the confidence intervals for rats and mice; however, the stratified analysis reduces the confidence interval around the human estimates at all severity levels. There appears to be important differences between the levels of each variable.

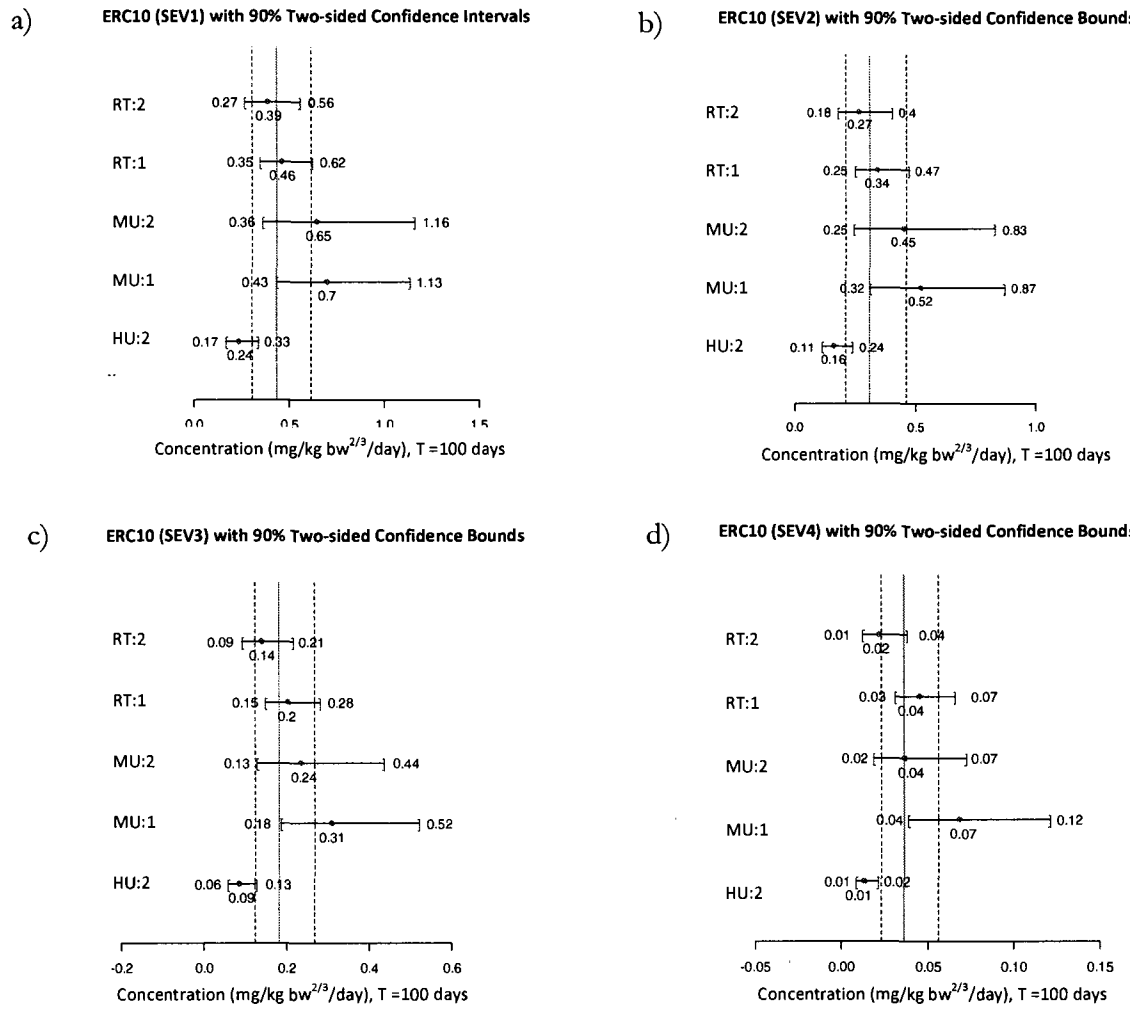


Figure 4.20a-d: Comparison of pooled versus stratified analysis with the cumulative odds model where the intercept is stratified by animal species and the concentration parameter is stratified by age. ERC10 estimates by animal species and age with 90% two-sided confidence intervals for severity level 1 (a), 2 (b), 3 (c) and 4 (d). Vertical lines represent ERC10-T100 for pooled analysis and horizontal lines represent ERC10-T100 for stratified analysis. Note. HU, humans; RT, rats; MU, mice; 1, young animals (≤30 days of age); 2, mature animals (>30 days of age) or adult humans (≥18 years of age).

4.8B Data Review for Outliners

CatReg was used to generate a plot (Figure 4.21) of the generalized deviance residuals versus the observation number. There does not appear to be any one stratum that is poorly described by the exposure-response curve. The observation with the largest deviance falls within the mature mice stratum. This observation, assigned to severity level 4, is from a study by Menino et al. (1986) where the exposure concentration was 0.0873 mg/kg $bw^{2/3}$/day and the exposure duration was T=60 days. In other mature mice studies, similar

and lower levels of copper have not been associated with as severe a severity score. This particular study was conducted to examine the influence of dietary copper on reproduction, growth and the cardiovascular system. Reproductive endpoints including in vitro blastocyte formation and fertilization rate appear to be sensitive markers of copper deficiency in female mice.

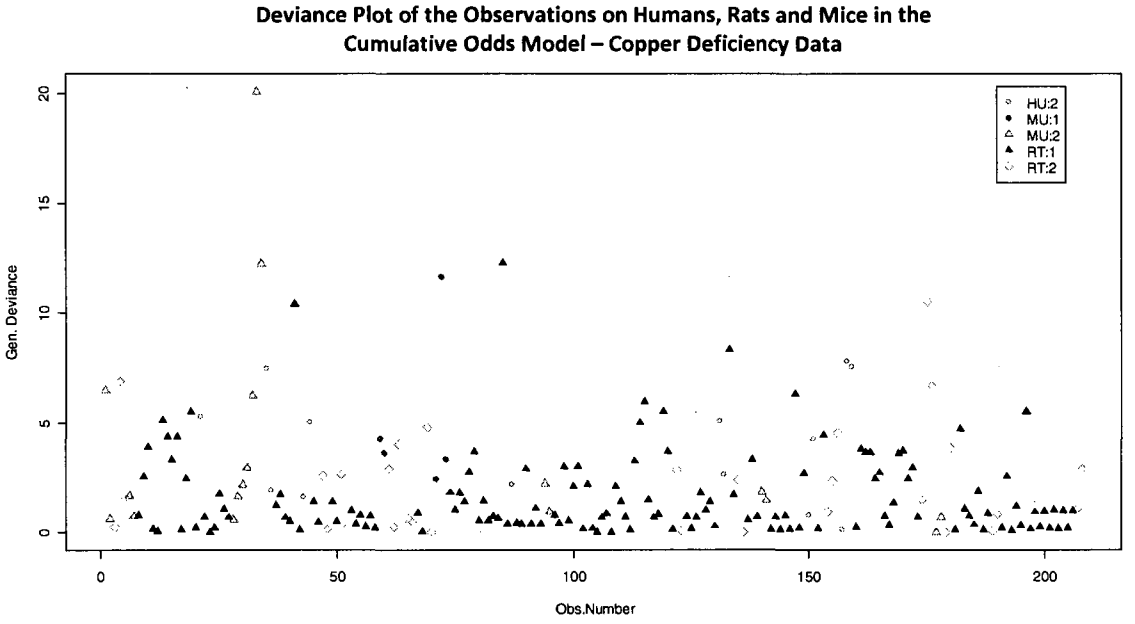


Figure 4.21: Deviance plot by observation #.

4.8C Model Fit

In the following section, the cumulative odds model defined in sections 4.8A to 4.8B was used to generate a series of plots for each stratum including a plot of the ERC10 line for severity level 2 or greater with two-sided 90% confidence intervals and a plot of all the ERC10 lines for each severity level. The plots focus on severity level 2 as the primary interest in the resulting exposure-response model is the acceptable range of oral intake, which has been defined by the ERC10 at severity level 2 or greater. A plot of the ERC10-T100 line for severity level 2 or greater (Figure 4.22) and a plot of the ERC10-T00 for all

severity levels (Figure 4.23) is presented below for the human strata only (dietary and drinking water strata). The plots for rats and mice can be found in Appendix J. As mentioned in section 4.5D, the ERC10 lines will not run through their corresponding observations. For the ERCq, as q increases from 0 to 1, the ERCq lines should become closer to their corresponding observations. As we have defined q equal to 0.10, the ERC10 lines for copper deficiency should fall above all corresponding observations.

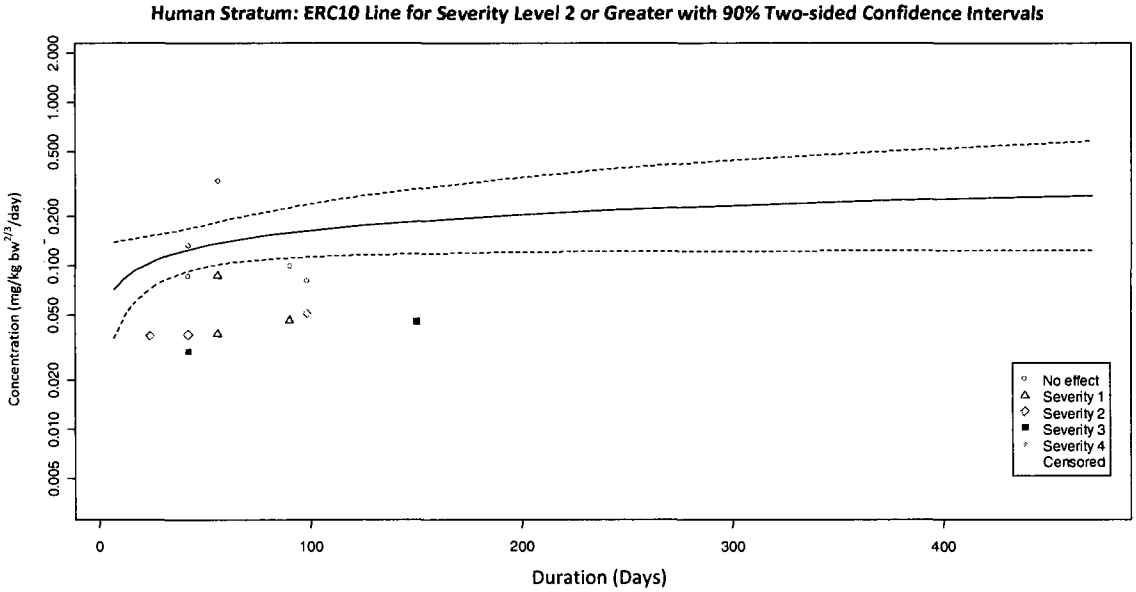


Figure 4.22: ERC10 line for severity level 2 or greater with 90% two-sided confidence intervals for the human stratum. Cumulative odds model with the probit link function transforms concentration (mg/kg $bw^{2/3}$/day) and duration (days) to the base 10. Intercept stratified by animal species and concentration by age.

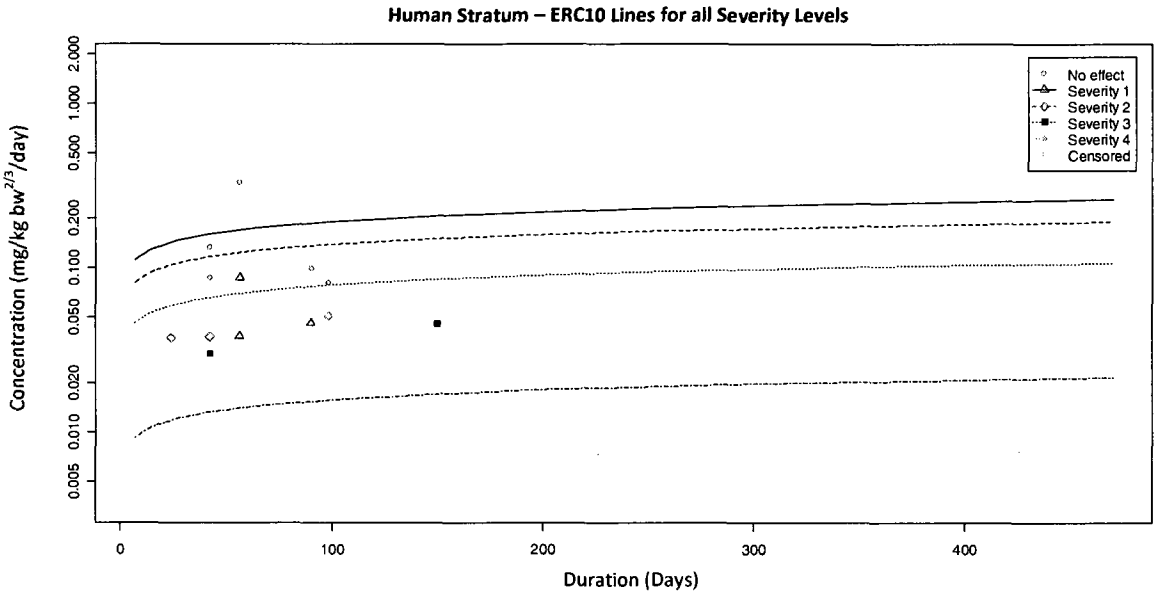


Figure 4.23: ERC10 line for all severity levels for the human stratum. Cumulative odds model with the probit link function transforms concentration (mg/kg $bw^{2/3}$/day) and duration (days) to the base 10. Intercept stratified by animal species and concentration by age.

The figures presented above and in Appendix J emphasize the minimal impact of duration in the exposure-response model. As mentioned previously, model fit can be evaluated by checking to see if the ERC10 line for each severity level is above its corresponding observations. In every strata, the plot of all the ERC10 lines shows that there is a large gap in the ERC10 lines between severity level 3 and severity level 4. For those strata that contain observations at severity level 4 (rat and mice stratum), the ERC10 line tends not to fall above its corresponding observations (i.e., observations at severity level 4). There are fewer observations at this severity level and for some strata there is a large amount of overlap in the assignment of severity levels 3 and 4. In every strata, the ERC10 line for severity levels 1 to 3 fall below their corresponding observations. The ERC10 lines for severity levels 1 and 2 are often extrapolated beyond the range of the available data in the rat and mice strata, whereas the ERC10 lines for severity levels 3 and 4 are often extrapolated beyond the range of the available data in the human stratum.

Variability in observed toxicity is demonstrated by overlap in the assignment of severity scores at common concentrations and durations of exposure. In the mature rat stratum, there is some variability among the data points assigned to severity levels 2 and 3. There is considerable variability in observed toxicity in the weanling rat stratum, particularly between severity levels 3 and 4. Furthermore, the concentration range between the ERC10 lines for severity level 0 and severity level 3 is very narrow. In the mature mice dietary stratum, there is some variability in the assignment of severity scores around 60 days of exposure. This variability in observed toxicity will be discussed in Part 5. Overall the cumulative odds model of the copper deficiency data with the intercept stratified by the animal species and the concentration parameter stratified by age appears to fit the data well for severity levels 1 to 3.

Figure 4.24 plots all the ERC10 lines by concentration and duration for each stratum at severity level 2 or greater. As mentioned earlier, it is difficult to visualize which ERC10 lines correspond do which strata; however, there are no options in CatReg that will improve the presentation of the plotting options available. The ordering of ERC10 lines from top to bottom in Figure 4.24 are as follows: weanling mice stratum, mature mice stratum, weanling rat stratum, mature rat stratum, and human stratum. This figure demonstrates that the animal species, route of exposure and age will impact how the exposure-response curve for copper deficiency is characterized. For example, the slope for the weanling rat and mice strata is greater than the slope of the mature rat and mice strata.

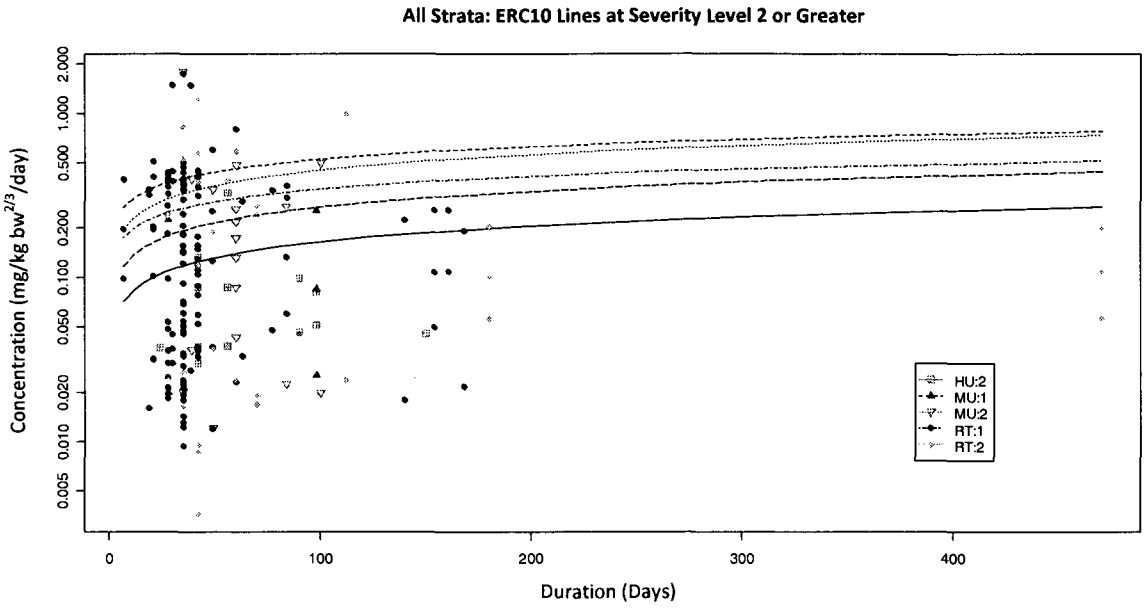


Figure 4.24: ERC10 line for severity level 2 or greater with 90% two-sided confidence intervals for all strata. Cumulative odds model with the probit link function transforms concentration (mg/kg $bw^{2/3}$/day) and duration (days) to the base 10. Intercept stratified by animal species and concentration parameter by age.

4.8D Final Estimates

As duration did not have a statistically significant effect in the exposure-response model, it will not be included in the final model Table 4.59 presents the ERC10-T100 estimates for severity level 2 or greater by animal species and age. After accounting for an approximate of surface area, the ERC10-T100 for severity level 2 or greater is approximately 2.8 times greater in mice than humans and 1.6 times greater in rats than humans. There is a difference of only 0.16 mg/kg $bw^{2/3}$/day between the ERC10-T100 for mature mice and rats and a difference of only 0.17 mg/kg $bw^{2/3}$/day between the ERC10-T100 for young mice and rats. Weanling rats and mice appear to be slightly more sensitive to copper deficiency than mature rats and mice. The difference between the ERC10-T100 estimates for rats, mice and humans is much less for copper deficiency than for copper excess. This will be discussed in Part 5.

Table 4.59: ERC10-T100 with 90% Confidence Intervals (CI) for Severity Level 2 or Greater by Animal Species and Age – Cumulative Odds Model* of the Copper Deficiency Data

Data Included in the Analysis	*ERC10-T100 (90% CI)*
Human	0.1385 (0.1056, 0.1816)
Young Rat	0.2793 (0.2283, 0.3417)
Mature Rat	0.2194 (0.1598, 0.3112)
Young Mice	0.4448 (0.2145, 0.6788)
Mature Mice	0.3816 (0.1598, 0.3012)

* Cumulative odds model uses the probit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log transformed. Intercept stratified by animal species and concentration stratified by age. Note. Young, young animals (≤30 days of age); mature, mature animals (>30 days of age) and adult humans (≥18 years of age).

SECTION 4.9 UNRESTRICTED CUMULATIVE MODEL – COPPER DEFICIENCY

As discussed in section 4.6, unlike the cumulative odds model (Equation 1), the unrestricted cumulative model (Equation 2) does not assume that the ERCq lines are parallel, as separate coefficients for C and T are estimated for each severity level (US EPA, 2000). Modeling the data with this more complex model allows us to evaluate whether the similar model (the cumulative odds model) is adequate to describe the exposure-response data.

4.9A Assumption of Parallelism

Table 4.60 presents the parameter estimates, standard errors, z-test statistics and p-values for the unstratified and unrestricted cumulative odds model of the copper deficiency data.

Table 4.60: Parameter Estimates, Standard Errors, Z-test Statistics and P-values for Copper Deficiency Studies Using the Unrestricted Cumulative Model*

Parameter	*Estimate*	*Std. Error*	*Z-test*	*P-value*
SEV1	-0.1233	1.0058	-0.1226	0.9024
SEV2	-0.7345	0.9855	-0.7453	0.4561
SEV3	-3.0609	0.9526	-3.2133	0.0013
SEV4	-4.3038	1.1636	-3.6987	0.0002
LG10CONC:SEV1	-7.6330	1.4353	-5.3180	<0.0001
LG10TIME:SEV1	-3.8440	1.0522	-3.6533	0.0003
LG10CONC:SEV2	-4.1729	0.7222	-5.7785	<0.0001
LG10TIME:SEV2	-1.9647	0.7577	-2.5930	0.0095
LG10CONC:SEV3	-2.1619	0.3133	-6.8996	<0.0001
LG10TIME:SEV3	0.3307	0.5548	0.6070	0.5438
LG10CONC:SEV4	-0.3307	0.3676	-0.8996	0.3683
LG10TIME:SEV4	1.4904	0.5586	2.6682	0.0076

* Unrestricted cumulative model uses the probit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log10 transformed. Note. SEV, severity level; LG10, log transformed to base 10; CONC, concentration coefficient; TIME, duration coefficient.

To test whether the similar model, the cumulative odds model (used in Section 4.8), is adequate to describe the data, there is a need to test whether the parameter estimates (i.e., concentration and duration) at each severity level in the unrestricted cumulative model are equal. The null hypothesis is that for the unrestricted cumulative model, the dose and duration parameters for severity level 1, 2, 3 and 4 are equal. The results of the Wald-type chi-square test for the equality of the concentration and duration parameters across severity levels 1 to 4 are presented in Table 4.61. The p-value was significant ($p<0.0001$), indicating that that the parameter estimates for concentration and duration for each level of severity are statistically different; and therefore it would be more appropriate to use the unrestricted cumulative model.

Table 4.61 Test for Equality of Concentration and Duration Parameters across Severity Levels 1 to 4

Chi-square	*df*	*P-value*
57.9365	6	<0.0001

Analysis uses the unrestricted cumulative model with the probit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log10 transformed.

4.9B Model Selection – Stratification Options

Using the unrestricted cumulative model with five levels of severity did not allow any model parameters to be stratified. Error messages in CatReg indicated that too many parameters were being calculated for the amount of data available. The model could be left unstratified or the number of severity scores could be reduced. A series of severity score combinations are presented in Appendix K. All models that use more than two severity levels result in incorrectly ordered parameter estimates. As with the copper excess data, a two-level severity model will be defined where observations originally scored a 0 or 1 will now correspond with severity level 0 and observations originally scored a 2 to 4 will now correspond with severity level 1.

The AIC from a series of models were compared to look at the impact of the animal species on the intercept, concentration and duration parameters (Table 4.62).

Table 4.62: AIC Comparison of Eight Modeling Options Defined by the Stratification of the Intercept, Concentration and/or Duration Parameter by Animal Species

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1	--	--	--	126.2938
2	Animal Species	--	--	95.9610
3		Animal Species		96.9844
4			Animal Species	96.7799
5	Animal Species	Animal Species		92.4404
6		Animal Species	Animal Species	93.61907
7	Animal Species		Animal Species	99.2822
8	Animal Species	Animal Species	Animal Species	96.3420

All eight models use the two-level severity model with the probit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log10 transformed.

Model 5, where the intercept and concentration parameter are stratified by the animal species, is associated with the lowest AIC. The effect of the animal species on the intercept ($p<0.0001$, $df=2$) and the concentration parameter ($p<0.0001$, $df=2$) is significant. The difference between the deviance of model 5 and model 2 was tested against a chi-square distribution with 2 degrees of freedom ($df_{model5} = 6$, $df_{model2} = 4$). The difference between the deviances of the two models (85.9610-78.4404=7.5206) is significant ($p<0.0234$, $df = 2$).

Table 4.63 presents the AIC for four models where the intercept, concentration and duration parameter was stratified by age. In every model, the intercept and concentration parameters are stratified by animal species.

Table 4.63: AIC Comparison of Four Modeling Options – Effect of Age on the Intercept, Concentration and Duration Parameter

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1	Animal Species	Animal Species		92.4404
2	Animal Species Age	Animal Species		95.32234
3	Animal Species	Animal Species Age		94.99044
4	Animal Species	Animal Species	Age	93.08414

All four models use the two-level severity model with the probit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) were log10 transformed.

The AIC did not decrease when the intercept, concentration or duration parameter was stratified by age. To look at the effect of sex, three categories were defined including 'males', 'females' and 'both'. Table 4.64 presents the AIC for four models where the intercept, concentration or duration parameter was stratified by age. In every model the intercept and the concentration parameter are stratified by the animal species. While model 4 is associated with the lowest AIC, there is only a negligible change in the AIC compared to model 1.

Table 4.64: Effect of Sex (Both, Males & Females) on the Intercept, Concentration or Duration Parameter – AIC Comparison of Four Modeling Options

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1	Animal Species	Animal Species		95.96104
2	Animal Species Sex	Animal Species		98.2945
3	Animal Species	Animal Species Sex		99.59772
4	Animal Species	Animal Species	Sex	95.39459

All four models use the two-level severity model with the probit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log10 transformed.

Stratifying the exposure-response model by a two-level sex variable does not decrease the value of the AIC (Table 4.65).

Table 4.65: Effect of Sex (Males & Females) on the Intercept, Concentration and Duration Parameter - AIC Comparison of Four Modeling Options

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1	Animal Species	Animal Species		90.08311
2	Animal Species Sex	Animal Species		92.9569
3	Animal Species	Animal Species Sex		93.2390
4	Animal Species	Animal Species	Sex	91.0080

All four models use the two-level severity model with the probit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log10 transformed.

Table 4.66 presents the AIC for four models where the intercept, concentration and/or duration parameter were stratified by solubility (low solubility versus high solubility). In each model, the intercept and the concentration parameter are also stratified by animal species. Model 4 is associated with the lowest AIC; however, the effect of solubility on the duration parameter is not significant (p=0.6839, df=1).

Table 4.66: Effect of Solubility on the Intercept, Concentration and Duration Parameter - AIC Comparison of Four Models

Model #	*Intercept*	*Concentration*	*Duration*	*AIC*
1	Animal Species	Animal Species	--	95.96104
2	Animal Species Solubility	Animal Species	--	96.4129
3	Animal Species	Animal Species Solubility	--	96.2203
4	Animal Species	Animal Species	Solubility	94.38039

All 4 models use the two-level severity model with the probit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) were log10 transformed.

The series of analyses up until this point have determined the following: the unrestricted cumulative model is too complex to consider all five severity scores (severity

scores 0 to 4) in the data; the unrestricted cumulative model with only three levels of severity is still too complex to consider different stratification options for the intercept, concentration and duration parameter; and finally, when the exposure-response model is defined by a two-level severity model a series of model comparisons determined that the model best describes the variability in the database when the intercept and the concentration parameter are stratified by the animal species. Table 4.67 presents the parameter estimates, the associated standard errors, Z-test statistics, and p-values for the two-level severity model. While duration is not significant in the final model, it will be retained in the analysis for the series of plots presented in section 4.9D. As mentioned in section 4.8, removing duration from the exposure-response model collapses all the data together at one time point, which reduces the ability to evaluate the degree of overlap in the assignment of severity scores. Furthermore, CatReg will not plot ERC10 lines when there is no variability in the duration of exposure (i.e., T=1).

Table 4.67: Parameter Estimates, Standard Errors, Z-test Statistics and P-values for the Two-level Severity Model* of the Copper Deficiency Studies

Parameter	*Estimate*	*Std. Error*	*Z-Test*	*P-value*
SEV1	-8.7732	2.5306	-3.4669	0.0005
HU:INTERCEPT	0	0	NA	NA
MU:INTERCEPT	-60.7520	2.9097	-20.8794	<0.0001
RT:INTERCEPT	4.8757	2.4439	1.9950	0.0460
HU:LG10CONC	-7.2978	1.8982	-3.8446	0.0001
MU:LG10CONC	-85.8496	2.1030	-40.8223	<0.0001
RT:LG10CONC	-5.2190	0.8918	-5.9641	<0.0001
LG10TIME	-0.5074	0.49991	-1.0166	0.3093

*Two-level severity model uses the probit link function. Intercept and concentration parameter stratified by the animal species. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log10 transformed. Note. SEV, severity level; LG10, log transformed to the base 10; CONC, concentration coefficient; TIME, duration coefficient; HU, human; MU, mice; R, rat

Figure 4.25 presents the results of the pooled and stratified analyses. The vertical line represents the pooled ERC10-T100 with two-sided 90% confidence intervals. The horizontal lines are the stratum-specific ERC10 estimates. There is a large difference between the ERC10-T100 for humans and the ERC10-T100 estimates for rats and mice.

There is also a large difference between the ERC10-T100 estimate for humans and the ERC10-T100 estimate for the pooled analysis. This emphasizes the importance of stratifying the exposure-response model by the animal species. The confidence interval for the mice stratum is extremely narrow. As most of the overlap in the assignment of severity scores in this stratum was between severity scores 0 and 1 and between severity scores 2 to 4, reducing the severity categories to two levels left no variability in the assignment of severity scores in the mice stratum. This will be discussed further in Part 5.

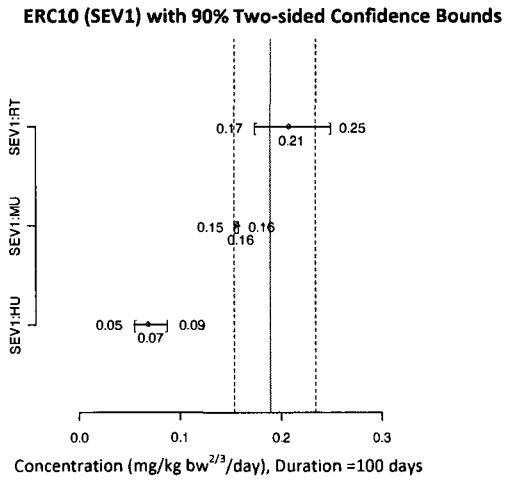


Figure 4.25: Two-level severity model with the probit link function. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) transformed (log10). Intercept and concentration parameter stratified by animal species.

4.9C Data Review for Outliners

CatReg was used to generate a plot (Figure 4.26) of the generalized deviance residuals versus the observation number. There does not appear to be any one stratum that is poorly described by the exposure-response curve.

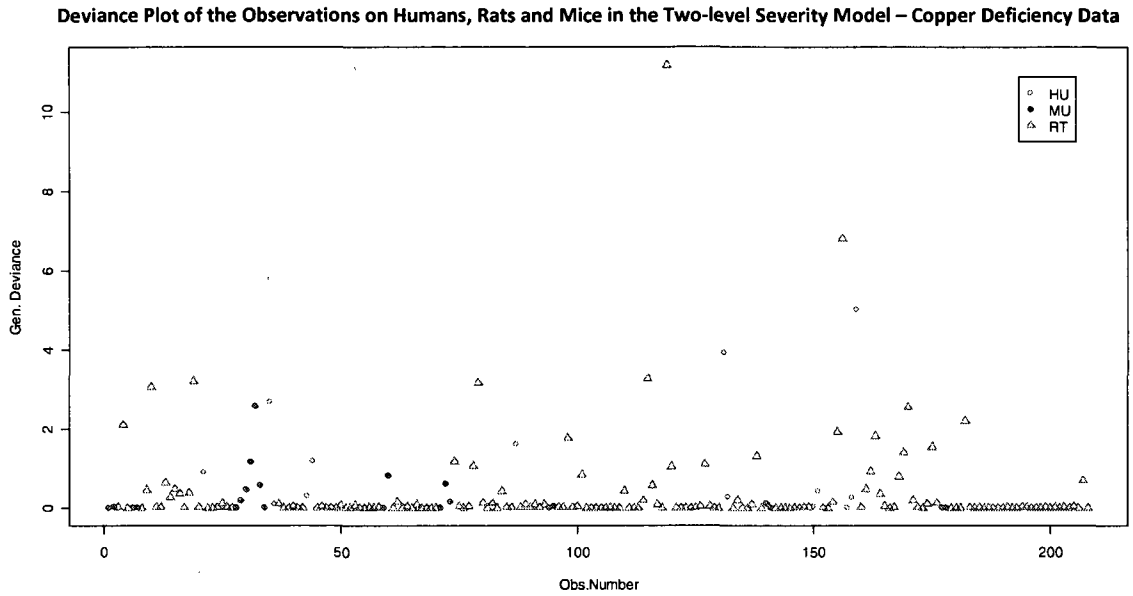


Figure 4.26: Two-level severity model uses the probit link function. Intercept and concentration parameter stratified by the animal species. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log10 transformed.

The data point with the highest residual deviance corresponds to an observation from Saari et al. (2002b). One of the observations from this study, which used a very deficient level of copper (0.05 mg/kg $bw^{2/3}$/day), was assigned a severity level 1 due to altered serum levels of copper. Changes in body weight, heart weight and hematocrit were only found in the group exposed to 0.02 mg Cu/kg $bw^{2/3}$/day. Had this study included a broader and more sensitive range of measures of copper deficiency (e.g., immune system dysfunction), this dose may have been assigned to a higher level of severity. Table 4.68 presents the ERC10-T100 for severity level 1 or greater for each stratum with and without this study. There is little change in the final estimates when this study is removed from the analysis.

Table 4.68: ERC10-T100 and Two-sided 90% Confidence Intervals (CI) With and Without Saari et al. (2002b)

Animal Species	With Saari et al. (2002b) *ERC10-T100 (90% CI)*	Without Saari et al. (2002b) *ERC10-T10 (90% CI)*
Human	0.07 (0.05, 0.09)	0.07 (0.05, 0.09)
Rats	0.21 (0.17, 0.25)	0.19 (0.17, 0.22)
Mice	0.16 (0.15, 0.16)	0.16 (0.15,0.26)

4.9D Model Fit

The two-level severity model defined in sections 4.9A to 4.9C was used to plot the ERC10 line for severity level 1 or greater with two-sided 90% confidence intervals for humans, rats and mice (Figures 4.27, 4.28, and 4.29, respectively). The series of figures on humans, rats and mice demonstrate that there is minimal impact of duration in the exposure-response model. The narrow confidence interval for the mice stratum is due to the absence of any variability in the assignment of severity scores at common concentrations and durations of exposure.

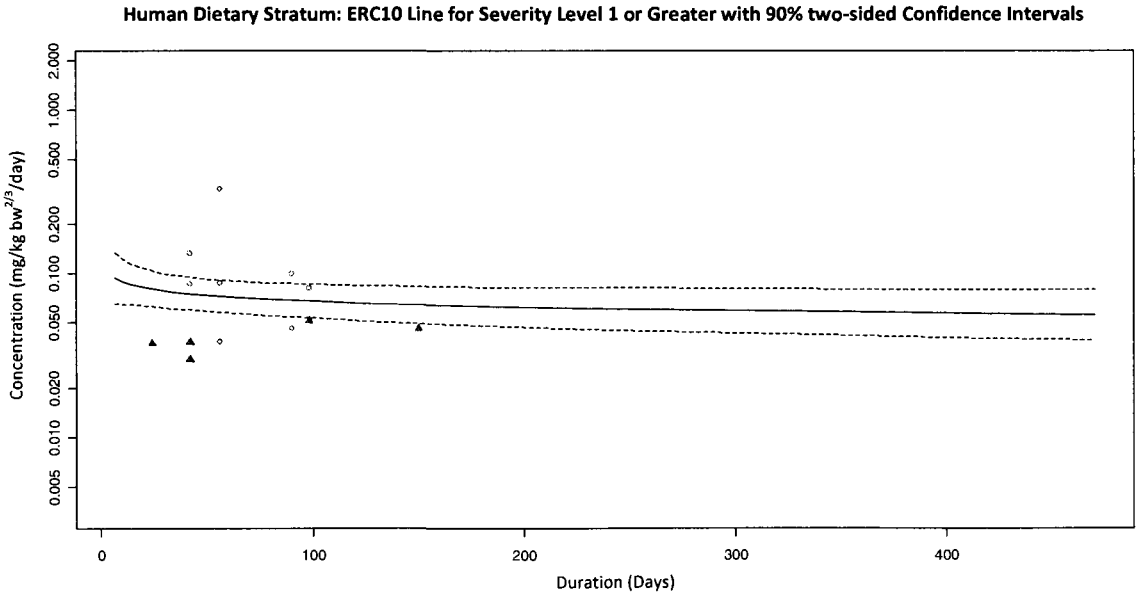


Figure 4.27: ○ = severity level 0, ▲ = severity level 1. Two-level severity model uses the probit link function. The intercept and concentration parameter are stratified by animal species. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log10 transformed.

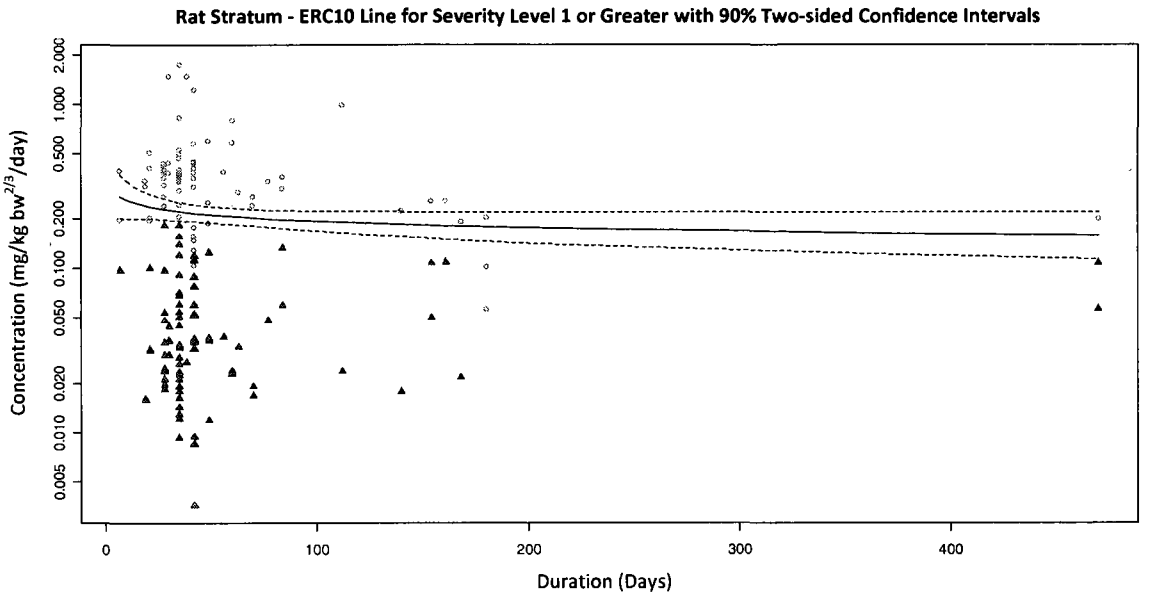


Figure 4.28: ○ = severity level 0, ▲ = severity level 1. Two-level severity model uses the probit link function. Intercept and concentration parameter stratified by the animal species. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log10 transformed.

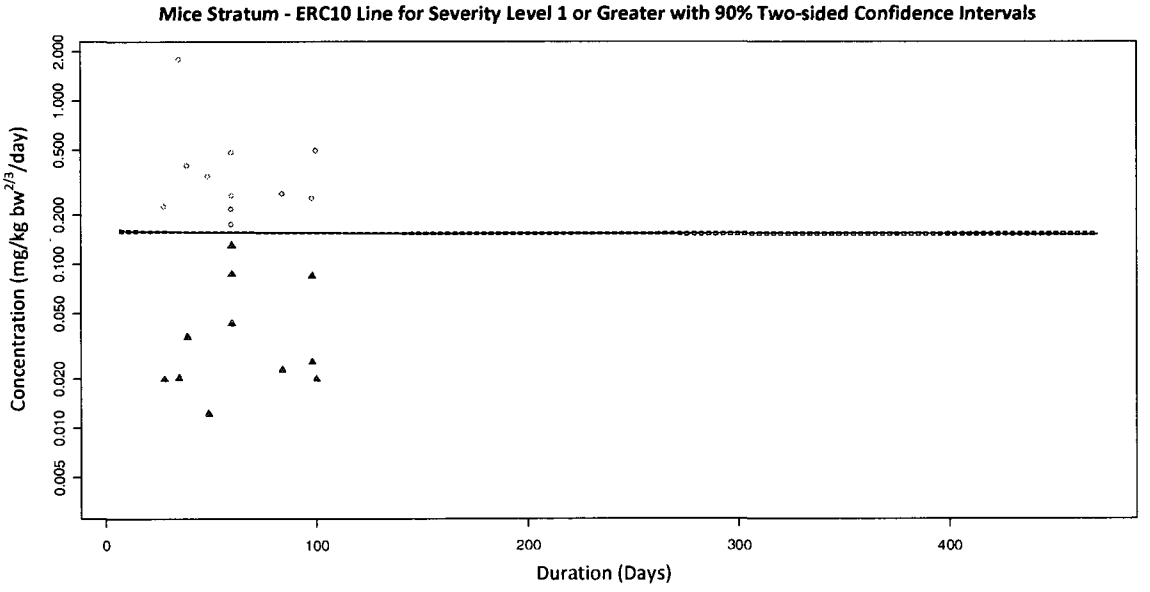


Figure 4.29: ○ = severity level 0, ▲ = severity level 1. Two-level severity model uses the probit link function. The intercept and concentration parameter are stratified by the animal species. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log10 transformed.

For the rat and human strata, while there is some variability in the assignment of severity scores 0 and 1, the ERC10 line for severity level 1 or greater is above all of its corresponding observations. The ERC10 line for severity level 1 or greater is also above all of its corresponding observations in the mice stratum. The two-level severity model, where

the intercept and the concentration parameter are stratified by the animal species, appears to fit the data well as all ERC10 lines are above their corresponding observations.

4.9E Final Estimates

As duration did not have a statistically significant effect on the severity of response, it will not be included in the final model. Table 4.69 presents the ERC10-T100 estimates for severity level 1 or greater by the animal species. After accounting for interspecies differences based on an approximate of surface area, the ERC10 for severity level 1 or greater is approximately 2.3 times greater in mice than humans and 3.3 times greater in rats than humans. The difference between the ERC10 estimates for rats and mice is less than 0.07 mg/kg $bw^{2/3}$ /day. When using the two-level severity model, the difference between the ERC10 estimates for rats, mice and humans is much smaller than what was seen with the cumulative odds model (section 4.8). Further comparisons of the results of the two modeling options for the copper deficiency data will be presented in section 4.10.

Table 4.69: ERC10-T100 with 90% Confidence Intervals (CI) for Severity Level 1 or Greater by Animal Species

Animal Species	*ERC10-T100 (90% CI)*
Humans	0.0689 (0.0545, 0.0872)
Rats	0.2259 (0.1980, 0.2577)
Mice	0.1565 (0.1557, 0.1575)

Two-level severity model uses the probit link function. Intercept and concentration parameter stratified by the animal species. Concentration (mg/kg $bw^{2/3}$/day) and duration (days) are log10 transformed.

4.10 COMPARISON OF THE CUMULATIVE ODDS MODEL AND THE TWO-LEVEL SEVERITY MODEL FOR COPPER EXCESS AND DEFICIENCY

In sections 4.5 and 4.6 and in sections 4.8 and 4.9, two models were used to analyze the copper excess and copper deficiency data: the two-level severity model and the cumulative odds model. This section will compare the final estimates, ERC10 lines and probability plots generated from both models on copper excess and deficiency.

Table 4.70 presents a comparison of the coefficient of determination for the two models on copper deficiency and the two models on copper excess. CatReg provides generalized analysis of variance and R^2 statistics for assessing the explanatory capacity of the exposure-response curve. The R^2 is computed by dividing the model deviance by the total deviance. There is a greater difference in R^2 between the two models on copper deficiency. For both copper excess and copper deficiency, the two-level severity model accounts for more variation in response. The large increase in the R^2 when using the two-level severity model is primarily a consequence of eliminating any variability in the data (overlap in the assignment of severity scores) between severity levels 0 and 1 and between severity levels 2 or greater.

Table 4.70: Comparison of the Coefficient of Determination (R^2) for Copper Deficiency and Excess - Two Level Severity Model versus the Cumulative Odds Model

Model	*Copper Deficiency*	*Copper Excess*
Two Level Severity Model	79.4%	65.4%
Cumulative Odds Model	38.6%	42.9%

Combining the severity scores into two levels for both copper excess and deficiency had a large impact on the final stratification options. Table 4.71 presents a comparison of the stratification options used in each model for copper deficiency and copper excess. For copper deficiency, while age had a significant effect in the cumulative odds model, it did not

have a significant effect in the two-level severity model. There are large differences between the stratification options in the two models of the copper excess data; however, the animal species, route of exposure, and age have a significant effect in both models.

Table 4.71: Comparison of Stratification Options for Copper Deficiency and Excess – Two-level Severity Model versus Cumulative Odds Model

Model Parameter	Stratified Variable	
	Copper Deficiency	*Copper Excess*
Two Level Severity Model		
Intercept	Animal Species	
Concentration	Animal Species	Specie, Route of Exposure, Age
Duration		Specie, Route of Exposure
Cumulative Odds Model		
Intercept	Animal Species	Animal Species, Route of exposure
Concentration	Age	
Duration		Age

Figure 4.30 presents the ERC10 line for humans at severity level 2 or greater using the cumulative odds model (Figure 4.30a) and the two-level severity model (Figure 4.30b) of the copper deficiency data. These figures demonstrate the there is a greater impact of duration in the cumulative odds model. However, duration did not have a significant effect in either model of the copper deficiency data. Using the coefficients for concentration and duration in each model we can estimate the concentration-duration relationship (C^nT). In the cumulative odds model, the resulting relationship was $C^{3.14}T$ and for the two-level severity model the relationship was $C^{7.81}T$. Concentration clearly has a greater impact than duration in the exposure-response model. Figure 4.31 presents the probability plot for humans using the cumulative odds model (Figure 4.31a) and the two-level severity model (Figure 4.31b) of the copper deficiency data. The probability plot in the two-level severity model is characterized by a much steeper slope than the cumulative odds model.

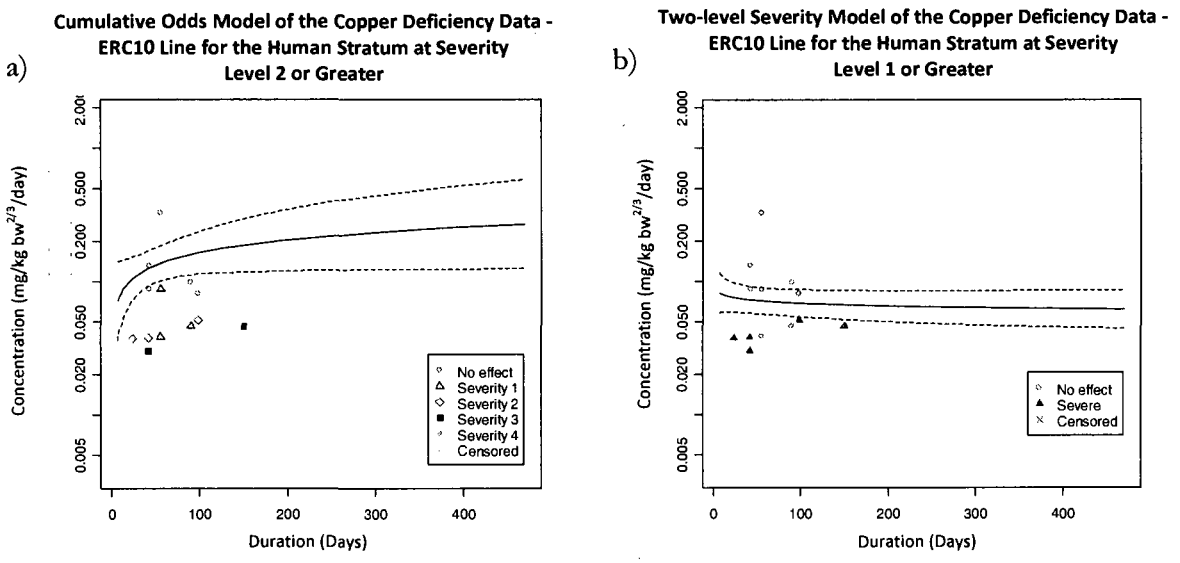


Figure 4.30a: ERC10 lines for humans at severity level 2 or greater with the cumulative odds model for copper deficiency.
Figure 4.30b: ERC10 lines for humans at severity level 1 or greater with the 2-level severity model for copper deficiency.

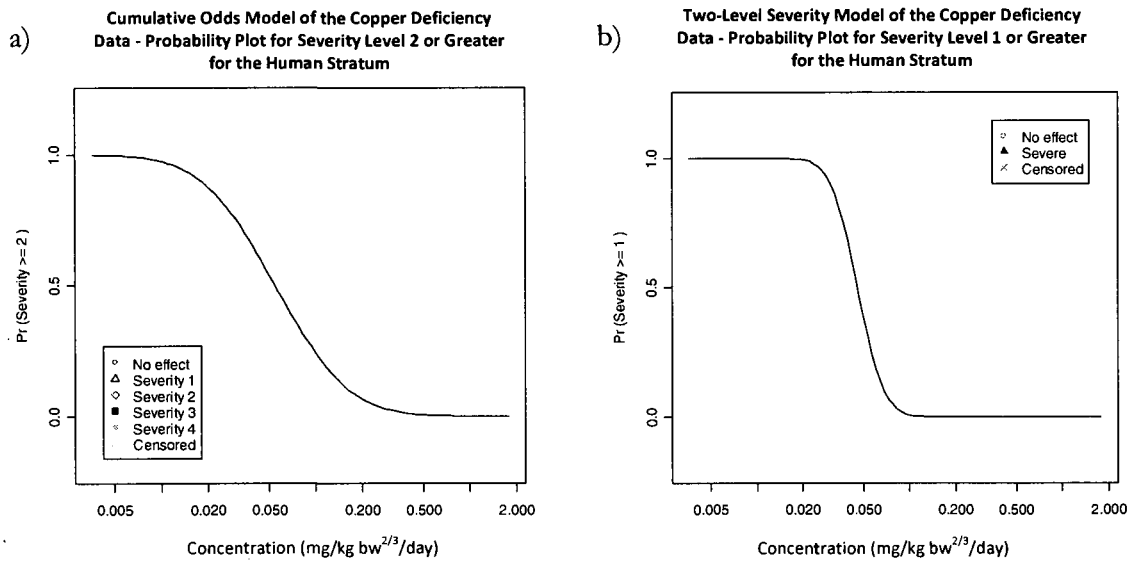


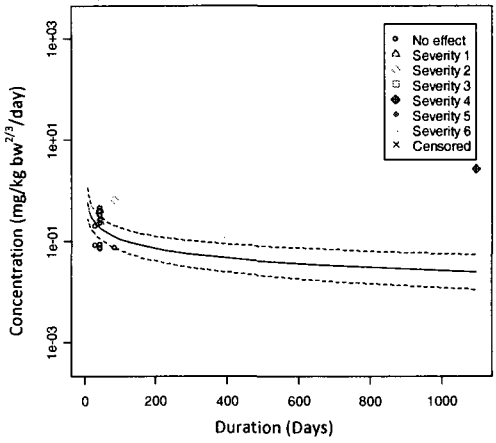
Figure 4.31a: Probability plot for humans at severity level 2 or greater with the cumulative odds model for copper deficiency.
Figure 4.31b: Probability plot for humans at severity level 1 or greater with the Two-level severity model for copper deficiency.

Figure 4.32 presents the ERC10 line for humans at severity level 2 or greater using the cumulative odds model (Figure 4.32a) and the two-level severity model (Figure 4.32b) of the copper excess data. While there is not much difference in the magnitude of the ERC10 lines, the confidence intervals around the ERC10 estimates for humans are wider in the two-

level severity model. Using the coefficients for concentration and duration in each model we can estimate the concentration-duration relationship (C^nT). In the cumulative odds model, the resulting relationship was $C^{1.6}T$ and for the two-level severity model the relationship was $C^{1.2}T$. Duration appears to have a greater impact in the two-level severity model.

Figure 4.33 presents the probability plot for humans using the cumulative odds model (Figure 4.33a) and the two-level severity model (Figure 4.33b) of the copper excess data. These figures demonstrate that the two-level severity model of the copper excess data (Figure 4.33b) produces ERC10 lines that have a much steeper slope than the ERC10 lines produced from the cumulative odds model (Figure 4.33a). There appear to be very important differences between the two models of the copper excess data.

a) Cumulative Odds Model of the Copper Excess Data - ERC10 Line for the Human Stratum at Severity Level 2 or Greater



b) Two-level Severity Model of the Copper Excess Data -ERC10 Line for the Human Stratum at Severity Level 1 or Greater

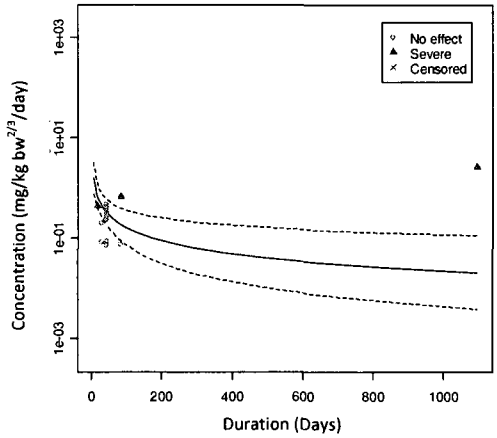
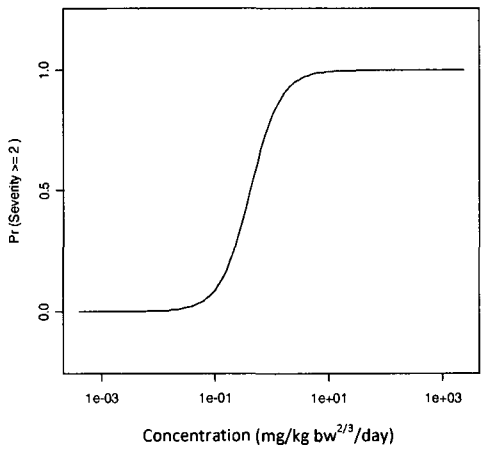


Figure 4.32a: ERC10 lines for humans at severity level 2 or greater with the cumulative odds model for the copper excess data. Figure 4.32b ERC10 lines for humans at severity level 1 or greater with the two-level severity model for the copper excess data.

a) Cumulative Odds Model of the Copper Excess Data - Probability Plot for Severity Level 2 or Greater for the Human Dietary Stratum



b) Two-Level Severity Model of the Copper Excess Data - Probability Plot for Severity Level 1 or Greater for the Human Dietary Stratum

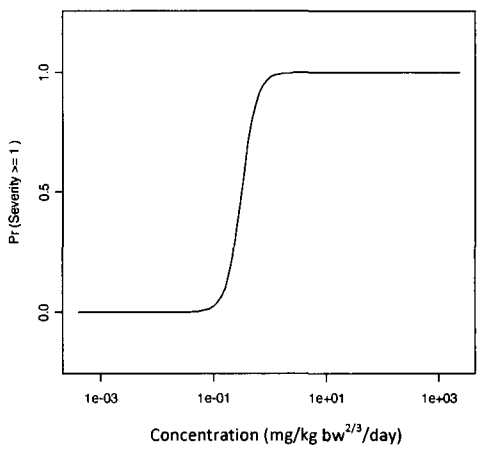


Figure 4.33a: Probability plot for humans at severity level 2 or greater with the cumulative odds model of the copper excess data. Figure 4.33b: Probability plot for humans at severity level 1 or greater with the two-level severity model of the copper excess data.

Table 4.72 presents a comparison of the ERC10 estimates between the two models for copper deficiency. For copper deficiency, the cumulative odds model results in more precautionary estimates for the ERC10. Table 4.73 presents a comparison of the ERC10-T100 estimates between the two models for copper excess. For copper excess, the cumulative odds model results in more precautionary estimates for the ERC10 for the human stratum only. The two-level severity model of the copper deficiency data produces

more precautionary estimates for rats and mice. A more precautionary estimate for copper excess is one that results in a lower estimate for the ERC10-T00 at severity level 2 or greater and a more precautionary estimate for copper deficiency is one that results in a higher estimate for the ERC10-T100 at severity level 2 or greater. There is a large difference between the final estimates for rats and mice between the two models for the copper excess data. The reason for the lower ERC10 estimate in the two-level severity model has to do with the fact that the ERC10 lines in the two-level severity model have a much steeper slope than in the cumulative odds model.

Table 4.72: Comparison of ERC10-T100 with 90% Two-sided Confidence Intervals (CI) for Copper Deficiency – Two-level Severity Model versus the Cumulative Odds Model

Data in the Analysis	*ERC10-T100* (90% CI)*
Two Level Severity Model	
Human	0.067 (0.06, 0.09)
Rat	0.23 (0.20, 0.26)
Mice	0.16 (0.16, 0.16)
Cumulative Odds Model	
Human	0.14 (0.11, 0.18)
Young Rat	0.28 (0.23, 0.35)
Adult Rat	0.22 (0.16, 0.31)
Young Mice	0.45 (0.22, 0.68)
Adult Mice	0.38 (0.16, 0.30)

*Concentration is defined mg/kg $bw^{2/3}$/day and duration is defined in days.

Table 4.73: Comparison of ERC10-T100 with 90% Two-sided Confidence Intervals (CI) for Copper Excess – Two-level Severity Model versus the Cumulative Odds Model

Data in the Analysis	*ERC10-T100 (90% CI)*
Two Level Severity Model	
Humans Dietary Studies	0.16 (0.07, 0.36)
Rats Mature Dietary Studies	2.07 (0.74, 5.76)
Rats Young Dietary Studies	1.94 (0.79, 4.79)
Mice Mature Dietary Studies	2.59 (0.73, 9.12)
Mice Young Dietary Studies	1.35 (0.91, 2.00)
Cumulative Odds Model	
Humans Dietary Studies	0.11 (0.07, 0.18)
Rats Mature Dietary Studies	7.22 (3.35, 15.55)
Rats Young Dietary Studies	2.12 (0.93, 4.83)
Mice Mature Dietary Studies	14.12 (6.28, 31.74)
Mice Young Dietary Studies	4.14 (1.35, 12.72)

*Concentration is defined mg/kg $bw^{2/3}$/day and duration is defined in days.

Figure 4.34 presents the human estimates for the ERC10, ERC25 and ERC50 at severity level 1 or greater (originally severity level 2 in the original scoring scheme) for the copper deficiency and copper excess data using the two-level severity model. The dose in mg/kg $bw^{2/3}$/day was converted to mg/day assuming an average human body weight of 70 kg. The same information is reported in Table 4.74.

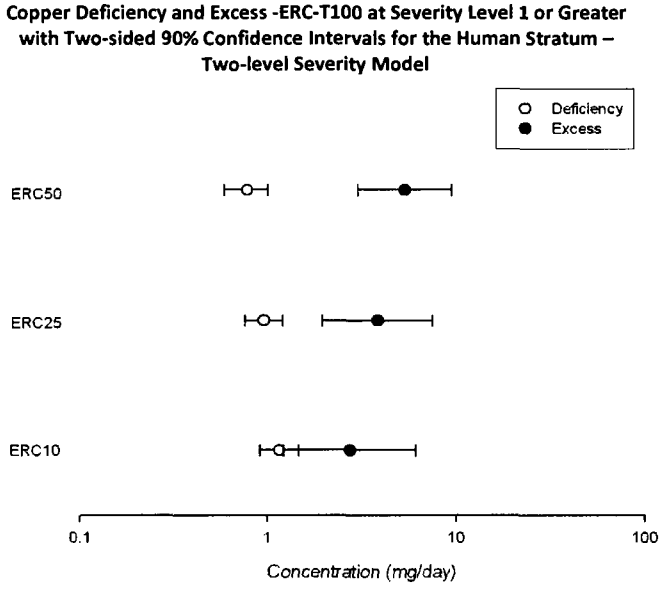


Figure 4.34: Two-level severity Model – ERC-T100 at severity level 1 or greater with two-sided 90% Confidence Intervals for the ERC25, ERC50 and ERC100. Concentration is defined in mg/day and duration is defined in days.

Table 4.74: ERC-T100 with Two-sided 90% Confidence Intervals (CI) for Severity Level 1 or Greater for the Human Stratum - Two-level Severity Model

	ERC-T100* (90% CI)	
Probability Level	***Deficiency***	***Excess***
ERC50	0.78 (0.59, 1.01)	5.33 (3.02, 9.41)
ERC25	0.96 (0.76, 1.21)	3.82 (1.95, 7.49)
ERC10	1.16 (0.92, 1.47)	2.74 (1.22, 6.13)

* Concentration is defined in mg/day and duration is defined in days

Figure 4.35 presents the ERC at 10, 25 and 50 percent probability levels for copper deficiency and copper excess using the cumulative odds model. The dose in mg/kg $bw^{2/3}$/day was converted to mg/day assuming an average human body weight of 70kg. The

same information is reported in Table 4.75. When using the cumulative odds model, the ERC10 estimates for copper deficiency and copper excess cross.

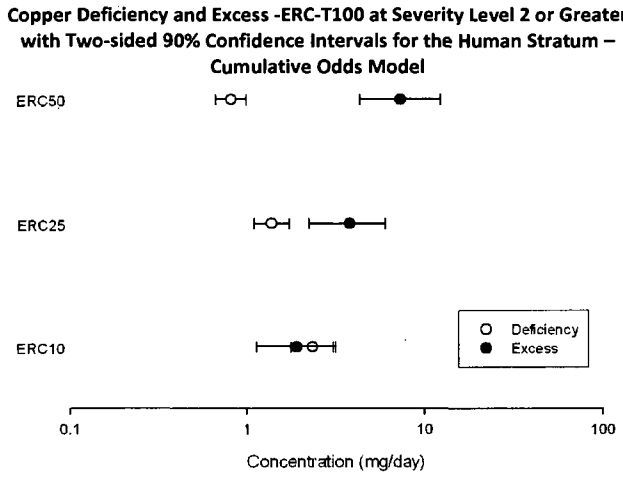


Figure 4.35: Cumulative odds model - ERC-T100 at severity level 2 or greater with two-sided 90% confidence intervals for ERC25, ERC50 and ERC100. Concentration is in mg/day and duration in days.

Table 4.75: ERC-T100 at Severity Level 2 or Greater with Two-sided 90% Confidence Intervals (CI) for the Human Stratum – Cumulative Odds Model

Probability Level	ERC10-T100 (90% CI)	
	Deficiency	*Excess*
ERC50	0.80 (0.66, 0.98)	7.13 (4.25, 12.06)
ERC25	1.36 (1.09, 1.71)	3.74 (2.21, 5.94)
ERC10	2.31 (1.75, 3.05)	1.88 (1.12, 3.13)

Concentration is defined in mg/day and duration is defined in days

In this section several comparisons have made between the use of the two-level severity model and the cumulative odds model. There is no one criterion that can be used to decide on which model to use. The large decrease in the model fit when using the two-level severity model is primarily a consequence of eliminating any variability in the data at severity level 2 or greater. One important difference between the two models of the copper deficiency data is the fact that the cumulative odds model found a significant impact of age on the concentration parameter, whereas the two-level severity model did not find that age had an effect on any of the model parameters. The absence of a statistically significant effect of age in the two-level severity model is likely a result of the reduced variability in scores at severity levels 2 or greater after they were combined. Compared to the two-level severity

model, the cumulative odds model results in more precautionary estimates for the ERC10-T100 as there is a greater difference in the space between the ERC10 curve and its corresponding observations at severity level 2 or greater.

For copper excess, there is a large difference in the final stratification options selected in the two-level severity model versus the cumulative odds model. While the stratification options are very different, the final human ERC10 line for severity level 2 or greater is more similar between the two models on copper excess compared to the two models on copper deficiency. The cumulative odds model will be selected to interpret the ERC10 estimates at severity level 2 or greater as this model accounts for greater variability in the database, and compared to the two-level severity model, produces more precautionary human estimates of the ERC10-T100 for copper deficiency and excess.

In the categorical regression analysis, the cumulative odds model defines an ERC10 estimate for severity level 2 or greater for copper deficiency (2.3 mg/day) that is higher than the ERC10 estimate for copper excess (1.9 mg/day). In Figure 4.36, the plots of the probability curves for severity level 2 or greater for both copper deficiency and copper excess have been placed on top of each other. The probability curves cross at approximately 2.1 mg Cu/day. Had we used the two-level exposure-response model, the midpoint between the ERC10 for copper deficiency and copper excess would have been approximately 2.0 mg Cu/day. It is important to note that as incidence data has not been used in this analysis, it complicates the interpretation of the final ERC10 estimates. If the data are on individual exposed subjects, then probability represents actual risk. If the data is only available at the dose group level, which is the case in this analysis, then p represents the probability that one would assign the dose group to a severity level s or greater (Hertzberg and Dourson, 1993). Part 5 will compare the ERC10 estimates for copper excess and deficiency with current

regulatory standards including the tolerable upper intake level and the recommended daily intake.

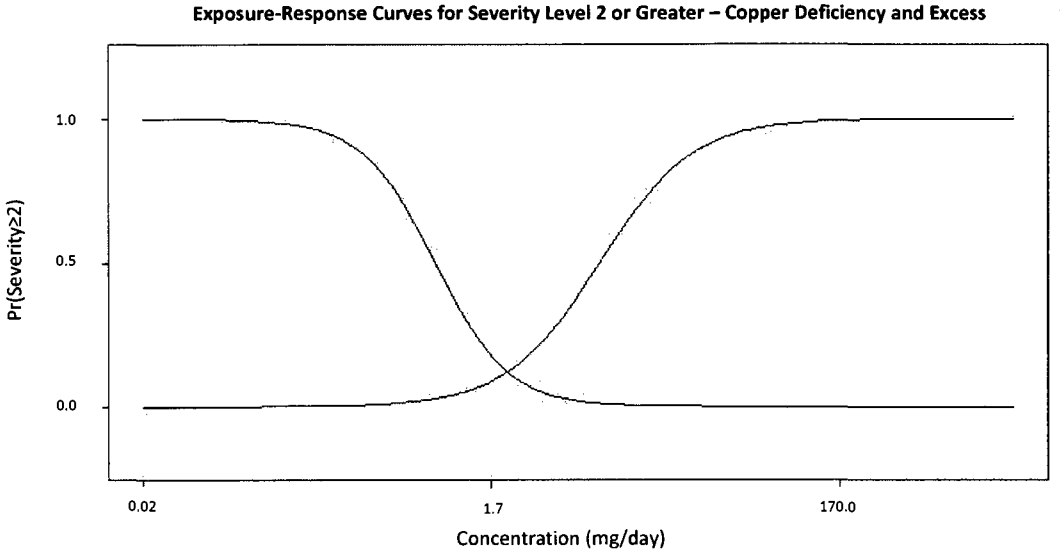


Figure 4.36: Probability curves for copper deficiency and copper excess for severity level 2 or greater. Concentration defined in mg/day and duration defined in days. The exposure-resonse data is modeled with the cumulative odds model. For copper deficiency, the probit link function is used and the intercept is stratified by animal species and the concentration parameter is stratified by age. For copper excess, the logit link function is used and the intercept is stratified by animal species and route of exposure and the duration parameter is stratified by age.

PART 5: DISCUSSION

This thesis illustrates how categorical regression, which combines data from different sources and uses a common severity scale, can be used as an analytical approach to critically evaluate data on copper excess and deficiency and define a range of dietary intakes that will meet the nutritional requirements of a healthy population as well as avoid adverse health effects from elevated copper intake. An expert panel from all appropriate scientific disciplines has developed a severity scoring system and a copper exposure-response database that optimizes the use of the available data on copper excess and deficiency. As illustrated in the analysis, considerable variability in the copper exposure-response database in terms of the study design, animal species, sex, and age could be accounted for by stratifying parameters in the exposure-response model. Whether we use a simple two-level severity scoring scheme or a more complex scoring scheme with up to six levels of severity, the midpoint between the resulting ERC10 estimates for copper deficiency and the ERC10 estimates for copper excess is approximately 2.0 mg Cu/day. These results suggest that current recommendations for copper including the recommended dietary intake (RDI) (0.9 mg/day) and the tolerable upper intake level (10 mg/day) may not protect the population from responses that might occur outside the limits of the homeostatic range, including increased markers of bone resorption, decreased concentration of immune cells, increased serum potassium, reduced superoxide dismutase activity, and altered cholesterol and triglyceride levels in the blood and liver. In order to ensure that recommended levels of copper are both safe and adequate, the range of current recommendations (0.9 to 10 mg Cu/day) could be narrowed to 1.8 to 3.1 mg Cu/day based on the lower limit of the 90% two-sided confidence interval around the ERC10 for copper deficiency and the upper limit of the 90% two-sided confidence interval around the ERC10-T100 for copper excess.

5.1 Database Review

The review of the current copper exposure-response database demonstrated that there are a limited number of studies on humans across a broad range of durations and levels of exposure. At this time, the human data alone are clearly inadequate to define an exposure-response curve for copper that takes into consideration a wide range of exposures and responses to copper excess and deficiency. The review of the copper database highlighted the degree of heterogeneity in terms of study design, animal species, sex, and age which suggested the need for a complex stratified exposure-response model.

5.2 Common Exposure Metric

Traditionally, animal data has been used in isolation to define a NOAEL, to which uncertainty factors are applied to transfer dose-response data expressed per kg body weight from animals to humans irrespective of the animal species used. In categorical regression, on the other hand, we can define a common dose metric based on body weight or surface area; incorporate data on rats, mice and humans; and generate different parameter estimates for the intercept, concentration and/or duration parameters for each animal species. When there is an absence of data in one stratum across a wide range of doses and durations of exposure, as well as across different levels of severity, data from another stratum can be used to support the stratum specific exposure-response curve through the use of dose-conversion factors.

In the categorical regression analysis, a common dose metric based on body weight or surface area is used for the same reasons that we begin with a common metric for the NOAEL approach. If there is less species variation when using a transformed dose, then predictions of toxic dose levels in humans from animal studies should be improved (Hertzberg et al., 1985). For copper excess, there was very little variation in the AIC across

the different dose metrics for common link functions and transformation options. For copper deficiency, the values for the AIC were similar across the different dose metrics, with the exception of a large increase in the AIC when using the dose metric defined in milligrams per day. Due to the fact that categorical regression allows for model parameters to be stratified by animal species, whether the common dose metric is defined based on bodyweight or an approximate of surface area does not necessarily have a large impact on the fit of the exposure-response model.

5.3 Modeling Options

The unrestricted cumulative model could not be used at this time to model the data on copper excess or copper deficiency. Even reducing the number of severity scores to three levels does not allow the model to account for differences in sensitivity to copper excess or deficiency based on the animal species, age, sex, and study design. However, had the unrestricted cumulative model been used, there would be so few observations across the different strata that high variability or extreme observations could greatly impact the shape of the exposure-response curves for each stratum and level of severity. We were able to model the copper excess and deficiency data with the cumulative odds model, where each severity level is assumed to have a common coefficient for concentration and duration. With this model, we were able to evaluate the degree of variability in the assignment of all severity scores at common concentrations and durations of exposure. It also allowed for an evaluation of the magnitude of the difference between the ERC10 lines between neighboring severity levels. For example, we were able to look at the difference between the ERC10 lines for severity levels 2 and 3 and compare it to the difference between the ERC10 lines for severity levels 3 and 4. For both copper deficiency and excess, the difference between the ERC10 lines between neighboring severity scores increased as severity increased. The

greatest difference in the ERC10 lines is between severity levels 3 and 4 for copper deficiency and between severity levels 4 and 5 for copper excess. We would not expect that the difference in concentration at neighboring severity levels to be equal. The severity levels fall on an ordinal scale which means that in terms of risk, the difference between severity levels 1 and 2 is not necessarily the same as the difference between severity levels 3 and 4.

In section 4.10, several comparisons were made between the use of the two-level severity model and the cumulative odds model. The difference between the two models in terms of the shape of the exposure-response curve and the model fit was much greater for the copper deficiency data compared to the copper excess data. For copper deficiency, there was more variability in the assignment of severity scores 2 to 4 compared to the copper excess data. Therefore, when severity levels 2 to 4 were combined in the two-level severity model, the variability in observed toxicity dropped dramatically. For copper excess, we saw that even when severity scores 0 and 1 were combined to form a new severity level 0 and severity scores 2 to 6 were combined to form a new severity level 1, there was still considerable variability between the new severity levels (severity scores 0 and 1).

The cumulative odds model was selected to interpret the ERC10 estimates at severity level 2 or greater for copper deficiency and excess as this model accounts for greater variability in the database and produces more precautionary estimates for copper deficiency and excess. As mentioned in section 4.10, a more precautionary estimate for copper excess is one that results in a lower estimate for the ERC10-T100 at severity level 2 or greater and a more precautionary estimate for copper deficiency is one that results in a higher estimate for the ERC10-T100 at severity level 2 or greater.

We were able to evaluate the assumption of parallelism with an unstratified model of the copper deficiency data, with five severity levels (i.e., severity levels 0 to 4). The

unrestricted cumulative odds model could not be stratified with the use of all five levels of severity as too many parameters had to be estimated for the amount of data available. In this unstratified model, the difference between the coefficient estimates for concentration and duration across all five severity levels was statistically significant, which indicates that the more complex model (the unrestricted cumulative model) would have been more appropriate for describing the copper deficiency data compared to the simplified cumulative odds model. However, we do not know whether this would be the case if we were able to stratify any of the model parameters by the animal species before evaluating the parallelism assumption.

For copper excess, there is a large difference in the final stratification options selected in the two-level severity model versus the cumulative odds model; however, the final human ERC10 line for severity level 2 or greater was more similar between the two models for copper excess compared to the two models for copper deficiency. The two-level severity model for copper excess appears to have a slightly steeper slope; however, the final ERC10-T100 estimates produced from the two models are similar. We were able to evaluate the parallelism assumption with an unstratified three-level severity model in section 4.6. The test indicated that the concentration and duration coefficients for each level of severity were not statistically different from each other and therefore the less complex model (i.e. cumulative odds model) could be used.

As the cumulative odds model for copper excess and copper deficiency was stratified and based on multiple levels of severity, it would have been optimal to define a stratified unrestricted cumulative model with five or more levels of severity; however, there is insufficient data available in the current copper database to support such a complex model.

5.4 Variability in Observed Toxicity

5.4A Within Study Variation in Response

One of the important advantages of using the cumulative odds model over the two-level severity model is the ability to visualize the variability in observed toxicity at common levels of exposure. As the database contains group level data and not incidence data, overlap in the assignment of severity scores is not due to variability among study subjects in their response to excess and deficient levels of copper within one experiment and dose group. The overlap in the assignment of severity scores can be a result of within study variation in response. For example, in the study by Hebert (1993), 2 of the 12 experiments were on mature mice exposed to excess levels of copper in their drinking water. A dose of 1,000 ppm of copper administered to males was assigned a severity level 0 as there were no statistically or clinically significant responses compared to controls. However, the same dose (i.e., 1,000 ppm) administered to females was assigned a severity level 3 due to altered brain and lung weights compared to controls.

5.4B Identification of Sensitive Endpoints

Sensitive endpoints can be identified in a categorical regression analysis by identifying potential outliners in the data. For example, in section 4.5C, two observations were identified as potential outliners in the residual deviance plot. One of these observations was from the study by Murthy et al. (1981) on adult rats, where a severity level 3 was assigned to an exposure group receiving 250 mg Cu/kg/day for 30 days. Responses associated with elevated copper intake included altered levels of dopamine, norepinephrine, and 5-hydroxytrytptamine in the brain. Several other experiments using even higher doses of copper for longer durations (e.g., 500 mg Cu/kg/day for 92 days) have only reported

responses associated with homeostatic adaptations to elevated copper (e.g., serum copper burden) (Hebert, 1993); however, these studies did not measure brain dysfunction.

The second observation identified in section 4.5C was from a human study by Turnlund et al. (2004). This study measured a broad range of indicators of copper excess generally not found in other studies. A severity level 2 was assigned to a dose group receiving 7.8 mg Cu/day for 18 days. Long-term high copper intake was found to impact indexes of copper status (e.g., serum copper and caeruloplasmin activity), an index of oxidant stress, and several indices of immune system functioning. Other studies on humans in the copper toxicity database have used similar exposure levels, including 6 mg Cu/day and 7.23 mg Cu/day for 42 days, but have only detected responses at lower levels of severity (severity level 0 and 1). These studies, however, were primarily focused on the effect of elevated copper status on markers of DNA damage and liver function, as well as biochemical markers of bone metabolism (Baker 1999a; 1999b; O'Connor 2003).

In section 4.8 and 4.9, one observation from the data on copper deficiency was identified as a potential outliner in a plot of all the observations by their residual deviance. One of these observations was from a study by Menino et al. (1986) on adult mice where a severity level 4 was assigned to a dose group receiving 3 ppm of copper in their feed for 60 days. This particular study was interested in the influence of dietary copper on reproduction, growth, and the cardiovascular system. Reproductive endpoints including in vitro blastocyte formation and fertilization rate appear to be sensitive markers of copper deficiency in female mice. In other adult mice studies, similar and even lower levels of copper have not been associated with a high level of severity.

5.4C Variation between Studies

Overlap in the assignment of severity scores can also be a result of variation between studies. Variability between studies in observed toxicity at common levels of exposure can be due to the fact that every study does not measure the same range of responses to copper toxicity. As traditional measures of copper imbalance have been shown to be insensitive to marginally excess and deficient exposures, there have been several studies that have investigated potentially more sensitive indicators of copper imbalance (e.g., markers of immune and nervous system dysfunction).

Plots of the ERC10 line along with all the observations within a defined stratum by concentration, duration and severity can also help identify the degree of variability in observed toxicity. For example, the plot of the ERC10 line for severity level 2 or greater for the adult rat dietary stratum that was defined by the cumulative odds model of the copper excess data showed some variability in observed toxicity between 150 and 180 mg Cu/kg $bw^{2/3}$/day at 15 days of duration (Figure G3, Appendix G). This group of overlapping severity scores was from the same study, conducted by Hebert (1993). Liver weight was found to decrease within a group of female rats in one experiment (1993) but not in another experiment on male rats being exposed to the same level of copper (i.e., 4,000 ppm for 15 days)

In another example in section 4.5C, a plot of the ERC10 line for severity level 2 or greater for the adult rat drinking water stratum showed some variability in the data between 7 and 20 days of duration (Figure G5, Appendix G). Four observations at severity level 0 were from the study by Haber (1993), where 7, 5, 25, and 17 mg Cu/day for 15 days was associated with severity level 0. The study focused on gross indicators of copper toxicity including body weight, clinical signs, renal histopathology, and survival. Other rat drinking

water studies have used lower levels of copper, and have found responses associated with higher levels of severity. For example, in the study by Goldschmith (2005), a dose of 12 mg Cu/day consumed in drinking water for 20 days was assigned a severity level of 4. This study reported several markers of brain function, including synaptic sensibility and facilitation capability. In a study by Lai et al. (2005), rats were exposed to 2.8 mg Cu/day in their drinking water. This dose group was assigned a severity level 3 based on markers of altered bone metabolism.

One of the limitations in the data available on copper excess and deficiency, which has been highlighted above, is the fact that there is considerable variability in the range of responses examined in different studies. The endpoint analysis in section 4.3 demonstrated the degree of variability in the types of responses associated with copper excess and deficiency. This accounts for some of the variability in observed toxicity. For example, when the cumulative odds model was used to plot the copper deficiency data for the mature rat stratum, there was considerable variability between severity scores 2 and 3 (Figure J1, Appendix J). The apparent variability in toxic response is a result of two very different studies (Bala et al. 1990 and Gomi et al. 1995) using a similar deficient levels of copper. In Bala et al. (1990), which uses a longer duration of exposure, the investigators reported a range of responses associated with the immune system from elevated copper intake that were assigned a severity level 3. In Gomi et al. (1995), which uses a shorter duration of exposure, they reported a range of responses including altered levels of ceruloplasmin and SOD activity from elevated copper intake that were assigned a severity level 2.

5.4D Model Fit

Plotting the ERC10 lines for all severity levels by concentration and duration is useful in determining whether each ERC10 line is well below (copper excess) or well above

(copper deficiency) its corresponding severity scores. For example, for the copper deficiency studies on young rats, there is considerable variability in observed toxicity across different studies and experiments; however, the ERC10 lines for severity levels 1 to 3 are well above their corresponding severity scores. For both copper excess and copper deficiency, the ERC10 line for severity level 4 or greater tends to run through its corresponding severity scores rather than above or below these observations. The exposure-response model does not appear to fit the data associated with higher severity scores as well as the data at lower levels of severity. The confidence interval about the ERC10 lines at these higher severity levels is wider than the confidence interval about the ERC10 lines at lower severity levels. This is likely due to the fact that there is currently a lack of data on copper deficiency at extreme levels of severity and there is considerable variability in the assignment of severity scores 3, 4, and 5 at common doses and durations of exposure among the copper excess data.

5.5 Stratification Options

5.5A Animal Species

In both models of the copper excess data (i.e., the two-level severity model and the cumulative odds model), smaller animal species appear to be less sensitive to copper toxicity. This is consistent with other studies where smaller species have been found to be less sensitive to toxic agents when doses are expressed per kilogram body weight (Boxenbaum, 1982; Davidson et al., 1986; Schneider et al., 2004). In Schneider's and associates' investigation of allometric principles for interspecies extrapolation they found that total clearance relative to body weight is higher for smaller species than for humans (Schneider et al., 2004).

Model selection and categorization of the severity scores influenced whether the animal species had an effect on the intercept, concentration and/or duration parameter. In

the two-level severity model of the copper excess data, the animal species had a significant effect on the concentration and duration parameter whereas in the cumulative odds model, the animal species had a significant effect on the intercept parameter. In both models, stratifying all parameters by animal species improved the explanatory power of the model; however, the effect of animal species on the intercept, concentration and duration parameter was not significant. If there was more data on humans and mice across different levels of exposure and different levels of severity, the animal species may have had a greater impact on the model parameters.

As there is a lack of observations assigned to severity level 1 and 2 for rats and mice and the majority of the data is associated with more extreme severity scores, when the observations for severity levels 2 to 5 are combined, the final ERC10 estimates will be heavily influenced by the higher severity scores. Information is "borrowed" from the data on rats and mice to define the slope of the exposure-response curve for humans through the use of a species conversion factor. There have been several animal studies that have used doses that overwhelm the homeostatic system that are unlikely to be representative of environmental exposures. Ethical considerations limit the use of extremely elevated or deficient levels of copper in experimental studies on humans. There is less data available at marginally excess and deficient levels of exposure among the studies on rat and mice compared to the studies on humans. In both models of the copper excess data (i.e., the cumulative odds model and the two-level severity model), there is a large difference in the ERC10 estimates after accounting for species differences on the basis of surface area. The difference between the ERC10 estimates across animal species would likely decrease if we had been able to use internal dose measurements reflecting the amount of copper reaching target tissues, rather than the amount of copper consumed. While sufficient information is

often provided in human studies on the amount of copper consumed, this information often has to be estimated in animal studies. In order to define the daily intake of copper in animal studies, we often need to estimate the bodyweight at the midpoint of the experiment and an average daily intake of feed. Assuming that body weight and feed consumption are unaffected by the experimental conditions in animal studies is a strong assumption that could contribute to the large difference in the magnitude of the ERC10 estimates for animals and humans. If we overestimated the amount of copper consumed in animal studies on copper excess the difference between the ERC10 estimates between humans and animals may be exaggerated. Had more information on the daily intake of feed or total amount of copper consumed been provided in these studies the resulting ERC10 estimate for rats and mice might decrease; however, lower ERC10 estimates among rats and mice would likely not affect the current estimates for humans, as categorical regression can account for differences between species beyond body weight or surface area by stratifying the model parameters. For example, if we modify the copper excess database so that the concentration of exposure for only dietary studies on rats and mice is reduced by 0.05 mg/kg $bw^{2/3}$/day and we rerun the analysis, there would be only a 0.01 mg/kg $bw^{2/3}$/day difference in the ERC10-T100 estimates for humans.

Compared to the analysis of the copper excess data, there is a smaller difference between the ERC10 estimates for humans, rats, and mice. Unlike copper excess, humans appear to be less sensitive to copper deficiency. Copper balance studies in humans have suggested that the body needs 1.5 mg Cu/day to replenish lost stores (Klevay, 1998). The National Research Council has estimated that rats require approximately 5 mg Cu/kg of feed (NRC, 1972) and that mice require 6 mg Cu/kg of feed (NRC, 1995). The estimated requirements for copper converted to mg/kg bw/day would be 0.02 mg/kg bw/day, 0.4

mg/kg bw/day, and 1.0 mg/kg bw/day for humans, rats, and mice, respectively (assuming humans, rats, and mice have an average body weight of 70 kg, 0.250 kg and 0.025 kg, respectively). It appears as though rats and mice require a greater amount of copper per kilogram body weight than humans. Using the cumulative odds model, the ERC10-T100 for severity level 2 or greater is approximately 0.03, 0.09 and 1.3 mg Cu/kg bw/day for humans, rats and mice, respectively. These results are consistent with the fact that humans require a lower average daily intake of copper per kilogram of body weight.

5.5B Duration

For humans, generalizations regarding the impact of duration of exposure from subchronic and chronic exposures cannot be made at this time due to a lack of data past 100 days of exposure. For rats and mice, duration does appear to have an important impact on the exposure-response curve for copper excess, as a lower ERC10 is required to produce the same response probability as duration increases. The influence of duration of exposure on the toxicology of metals depends on toxicokinetic factors. Some metals are considered as cumulative toxins, where the same dose given over different periods of time can accumulate in excretory organs (Hayes, 2007). Essential elements have been found to be excreted more efficiently, so that any dose that can be tolerated for a short period of time can also be tolerated for an extended period of time (Hayes 2007). In section 4.5E, we demonstrated that if we remove the duration parameter from the cumulative odds model of the copper excess data, there is little impact on the ERC10 estimate for humans at severity level 2 or greater. With duration in the model, the ERC10 is 1.83 mg/day, and without duration in the model the ERC10 increases to 2.02 mg/day.

Duration appears to have no effect on the exposure-response curve for the copper deficiency data. We may have seen an impact of duration had there been more studies with chronic exposure durations.

5.5C Route of Exposure

The route of exposure (drinking water versus feed) also had a significant effect in the exposure-response model. The problem with the data from drinking water studies on humans is that there are currently no observations that correspond with severity levels 1 through 3. Adverse effects have only been associated with severity level 0 and severity level 4. Furthermore, the gap between severity level 0 and severity level 4 is extremely narrow. For example, in one study 0.41 mg Cu/kg $bw^{2/3}$/day was associated with a severity level 0, whereas 0.58 mg Cu/kg $bw^{2/3}$/day was associated with a severity level 4. While these drinking water studies have measured markers of copper imbalance that could be influenced by subchronic or chronic exposures to excess levels of copper (e.g., hemoglobin, serum Cu burden, ceruloplasmin, and serum enzymes) the only responses associated with a severity level 4 have been recurrent gastrointestinal symptoms (e.g., nausea). Gastro-intestinal symptoms are likely only an acute response to the copper solution consumed daily, rather than chronic effects of long-term consumption. However, the removal of the drinking water studies on humans from the analysis did not appear to impact the precision of the human estimates for the dietary stratum, nor greatly influence the fit of the exposure-response model. The impact of route of exposure can be seen in rat and mice studies where there have been a greater number of studies using drinking water. These studies have measured a broad range of sensitive markers of copper excess. As expected, the ERC10 for the drinking water stratum for rats and mice is less than the dietary stratum, even after adding a habitual intake of copper that would be found in a typical rat or mice diet. Rats and mice appear to

be more sensitive to copper excess when the exposure is administered in drinking water, as compared to the same dose administered in the diet. The impact of the route of exposure for humans cannot be adequately assessed at this time. More studies on copper excess in humans are needed where copper is ingested via drinking water. Such studies should ideally include a broad range of sensitive markers of toxicity (e.g., markers of immune system dysfunction) and use a chronic duration of exposure.

5.5D Age

Mature rats and mice required a slightly more deficient dose to achieve the same 10% response probability as young rats and mice; however, the difference between mature and young animals in the ERC10 was less than 0.1 mg/kg $bw^{2/3}$/day for rats and less than a 0.06 mg/kg $bw^{2/3}$/day for mice. The difference was more pronounced for copper excess, where weanling rats and mice were also more sensitive than mature rats and mice. The results are consistent with other findings that have shown young rats absorbed copper in a concentration-dependent fashion with limited feedback control or saturability (Coudray et al., 2006; Varada et al., 1993).

5.5E Sex

In the cumulative odds model of the copper excess data, sex did not have a significant effect in the exposure-response model. Model fit did improve when the concentration parameter was stratified by sex in the two-level severity model of the copper excess data; however, the practical effects on the resulting ERC10 estimates for males and females were limited. In both models of the copper deficiency data (i.e., the cumulative odds model and the two-level severity model), the effect of sex on the intercept, concentration and/or duration parameter was not significant. For humans, this is consistent with the

finding that, on a body-weight basis, men and women have similar copper requirements (Johnson et al., 1992).

In animal studies, however, differences have been observed between males and females. There are currently very few observations in the copper toxicity database that are based on female rats. In terms of the drinking water studies, the majority of the data on humans has come from studies that have only focused on female subjects, or where the results have not been reported separately for males and females. While the majority of studies on mice are based on females, the majority of studies on rats are based on males. More studies are needed where both males and females are exposed to excess and deficient levels of copper and where the results are reported separately by sex.

The lack of an effect of sex in the exposure response-model could be due to inconsistencies found in the literature. In some studies males appear to be more sensitive to copper toxicity whereas in other studies females appear to be more sensitive. Although it has been suggested that the impact of sex on liver copper accumulation in rats depends on the strain used (Fuetealba et al., 2000), the current exposure-response model does not take into consideration the animal strain. Furthermore, the impact of sex has been shown to vary depending on the target organ of observed toxicity. At this time, there is insufficient data available to incorporate the target organ and animal strain in the exposure-response model.

5.5F Solubility

The present analysis was able to look at the effect of solubility in the exposure-response model. There was one study on weanling rats that examined the effect of copper excess that used a less soluble form of copper (Liu et al., 1986). Its removal from the analysis had little impact on the ERC10 estimate for weanling rats. For copper deficiency, there were several studies using less soluble forms of copper (Allen 1996; Bala 1990;

Davidson 1992; Gomi 1995; Hopkins 1995; Mao 1999; Rock 1995; Wang 1996; Rayssiguier 1993; Ajayi 2005; Klaahsen 2007). In the cumulative odds model, solubility had a significant effect on the intercept term; however, its inclusion in the model did not improve the model fit. There was also a very small difference between the ERC10 estimate for the low solubility strata and the high solubility strata among rats. The effect of solubility was greater for mice where the ERC10 estimate for less soluble forms of copper was higher (thus reflecting a less deficient dose) than the ERC10 estimate for more soluble forms of copper. While there is minimal difference between these ERC10 estimates, the results are consistent with the fact that absorption tends to be higher for soluble or ionic forms of copper compared to less soluble or insoluble mineral forms of copper (NRC, 1989).

5.6 Comparison of Results with Current Regulatory Values

As mentioned in section 4.10, the cumulative odds model defines an ERC10 estimate for severity level 2 or greater for copper deficiency (2.4 mg/day) that is higher than the ERC10 estimate for copper excess (1.8 mg/day). The probability curves (i.e., the exposure-response curve) for copper deficiency and excess have been found to cross at approximately 2.1 mg Cu/day (Figure 4.36). It is important to note that as incidence data has not been used in this analysis, the interpretation of the final ERC10 estimates is somewhat complicated. If data on individual exposed subjects were used, then probability would represent actual risk. If the data is only available at the dose group level, which is the case in this analysis, then p represents the probability that one would assign the dose group to a severity level s or greater (Hertzberg and Dourson, 1993).

The application of categorical regression to characterize the exposure-response curve for copper does not result in the originally theorized U-shaped exposure–response curve, but rather a more V-shaped exposure-response. The categorical regression models do not define

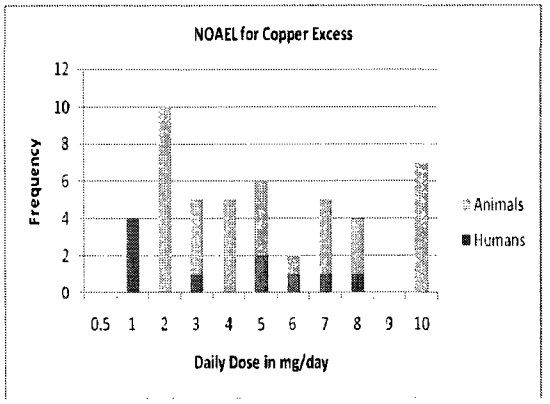
an acceptable range of oral intake, but rather a single optimal intake level. At 2.1 mg Cu/day one still might be at risk of responses associated with severity level 2 or greater from both copper excess and deficiency. Severity level 2 corresponds with early biological changes of accumulated copper that may or may not result in clinical signs of copper toxicity from excess and deficiency. In the copper toxicity database, responses that have been categorized as severity level 2 include: loss of Cu-dependent enzyme function, changes in blood cell number or function, reduced superoxide dismutase activity, and altered cholesterol and triglyceride levels in the blood and liver. If we use the lower confidence limit for the ERC10 for copper deficiency and the upper confidence limit for the ERC10 for copper excess, the boundary of the optimal intake level or the acceptable range of oral intake would be defined as 1.8 to 3.1 mg Cu/day.

Current recommendations for copper intake range from 0.9 mg Cu/day (recommended dietary intake) to 10 mg Cu/day (tolerable upper intake level) (Food and Nutrition Board, 2001). The ERC10 estimate that corresponds to where the probability curves for copper deficiency and copper excess cross, is several orders of magnitude below the tolerable upper intake level for copper (10 mg Cu/day) established by the Food and Nutrition Board (2001) of the U.S. National Research Council. One of the issues with the current upper intake level is that it is based solely on the NOAEL for markers of liver function that was identified in a single study (Pratt et al., 1985) without considering other markers of copper imbalance that may occur in other target organs. If for every study in the copper toxicity database, which considers a broad range of markers of copper toxicity, we had applied a traditional risk assessment approach where a NOAEL identified from a single study is divided by an uncertainty factor, we would likely end up with a reference dose that is more similar to the results generated in the categorical regression. This would be due

to the fact that the studies in the copper toxicity database consider less severe but still clinically important responses to elevate copper intake, whereas the tolerable upper intake level is based only on liver toxicity.

Figures 5.1a-b present a plot of all the NOAELs in the copper toxicity database from studies on copper excess. The highest dose within each experiment that was assigned a severity level 0 or a severity level 1 was considered a NOAEL. Two approaches to define an uncertainty factor for animals were considered. In Figure 5.1a, an uncertainty factor of 13 was selected to extrapolate from rodents to humans. This uncertainty factor was based on the largest difference between the intercept coefficients (background risk) for rodents and humans, which was generated from the cumulative odds model of the copper excess data where the intercept was stratified by animal species. Figure 5.1a demonstrates that the NOAEL approach would define a reference dose that could range from 1 to 10 mg Cu/day depending on the study selected. In Figure 5.1b, a traditional uncertainty factor of 100 has been used to extrapolate from rodents to humans and an uncertainty factor of 2 has been selected for humans. The European community used an uncertainty factor of 2 to define the tolerable upper intake level for copper in order to account for the potential variability in the normal population. Figure 5.1b also demonstrates that the traditional NOAEL approach would define a reference dose that is similar to what was estimated in the categorical regression analysis if all studies in the copper toxicity database were considered.

a)



b)

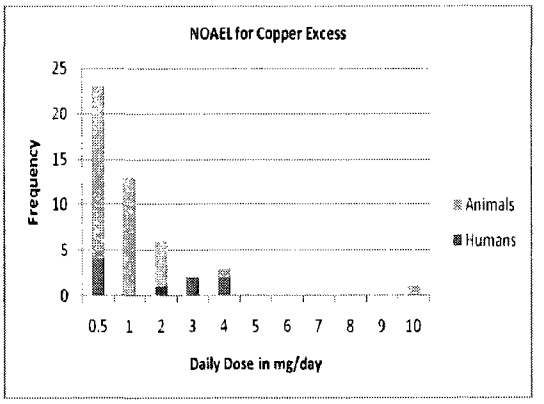


Figure 5.1a-b: NOAELs in the copper toxicity database. 5.1a NOAEL for animals divided by an uncertainty factor of 13. 5.1b NOAEL for animals are divided by an uncertainty factor of 100 and the NOAEL for humans are divided by an uncertainty factor of 2.

The current estimated average requirement (EAR) for copper is equal to 0.7 mg Cu/day (Food and Nutrition Board, 2001). The EAR is defined as the intake level for a nutrient at which the needs of 50% of the population will be met (Figure 5.2).

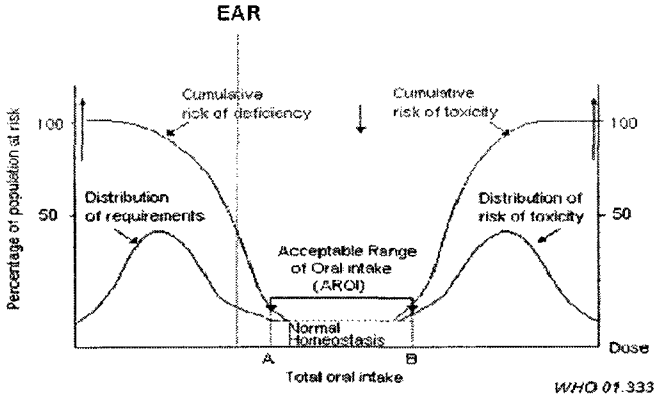


Figure 5.2: Theoretical Representation of the acceptable range of oral intake and the estimated average requirement (WHO, 2002). Reprinted by permission from the World Health Organization.

The EAR was set based on three studies. One study found that 0.4 mg Cu/day was not adequate to maintain levels of serum copper, ceruloplasmin and SOD activity in 8 of 11 young men. In the second study, 0.8 mg Cu/day did not result in a significant decline in serum copper, caeruloplasmin, or SOD activity. Based on these two studies it was decided that the copper requirement to maintain copper status in half of the individuals in a group is more than 0.4 mg/day but less than 0.8 mg/day. Data from these two studies was then used

to build a linear model, which suggested that half of these men would not maintain their copper status with a copper intake of 0.6 mg/day. The third study found that platelet copper concentration declined in 8 of 10 women given 0.6 mg/day, but increased with copper supplementation. While the EAR based on the first two studies was 0.6 mg/day, the third study suggested that 0.6 mg/day may be a marginal intake level in over half the population. Another increment was added to cover half of the population, resulting in an EAR of 0.7 mg Cu/day. The RDI, which is currently set at 0.9 mg/day, is defined as being equal to the EAR plus twice the coefficient of variation to cover the needs of 97% to 98% of the population.

There are several reasons why the ERC10 estimate for severity level 2 or greater defined in the categorical regression analysis is several orders of magnitude higher than the RDI. The approach used to define the EAR and the RDI only considers three studies on copper. On the other hand, the categorical regression approach takes into consideration more studies on copper deficiency, beyond the three copper balance studies used to set the EAR. For example, in the study by Kelley et al. (1995), which is included in the copper toxicity database, 0.7 mg Cu/day for 24 days was associated with severity level 2 due to a significant decrease in a range of markers of immune function. Baker et al. (1999b) found that 0.7 mg Cu/day for 42 days was associated with severity level 2 due to significant increases in a range of markers of bone resorption. In the study by Klevay et al. (1986), 0.8 mg Cu/day for 150 days was associated with severity level 3 due to increased plasma glucose levels and decreased insulin response, as compared to controls. In the study by Reiser et al. (1987), 0.9 mg Cu/day for 98 days was associated with severity level 2 due to statistically significant increases in plasma low-density-lipoprotein and statistically significant decreases in plasma high-density-lipoprotein.

Had a traditional risk assessment approach been applied to all the data in the copper toxicity database, including those studies described above, we would likely end up with a dose that is more similar to the results generated in the categorical regression analysis. Animal data is not often used in nutrition to set minimum requirements for humans. Unlike copper excess, where smaller animals are less sensitive to copper toxicity from excess, animals require a higher dose per kilogram body weight to maintain adequate copper stores. When the NOAELs from studies on animals are defined by mg/kg bw/day and then converted to mg/day based on an average human body weight (i.e. 70 kg), the resulting estimates are in excess of what would be considered typical for humans. For example, multiplying all the NOAELs for animals defined in mg/kg bw/day by 70 kilograms results in estimates ranging from 20 to 50 mg Cu/day. To use the animal data we could consider interspecies differences in sensitivity informed by the difference in background risk (intercept coefficients) between rodents and humans in the categorical regression model. The cumulative odds model was used to model the copper deficiency data where dose was defined by mg/kg bw/day and the intercept was stratified by animal species. A factor of 4 was selected based on the largest difference between the intercept coefficients (background risk) for humans, rats and mice. Each NOAEL for animals was divided by a factor of 4. The NOAEL for humans was multiplied by a factor of 1.3. To account for variation in the population when setting the RDI, a factor of 1.3 is typically applied to the estimated population mean requirement. Figure 5.3 demonstrates that if the traditional NOAEL approach was applied to all the studies on copper deficiency in the database, depending on the study selected, the adequate intake level would likely range from 6 mg Cu/day to 10 mg Cu/day. The purpose of displaying these NOAELs for copper excess and copper deficiency is to demonstrate that the study selected to define regulatory values such as the RDI or the

tolerable upper intake level will have a large impact on the resulting estimates. Unlike traditional methods used to set regulatory values, the categorical regression approach is able to incorporate all relevant studies in a combined analysis.

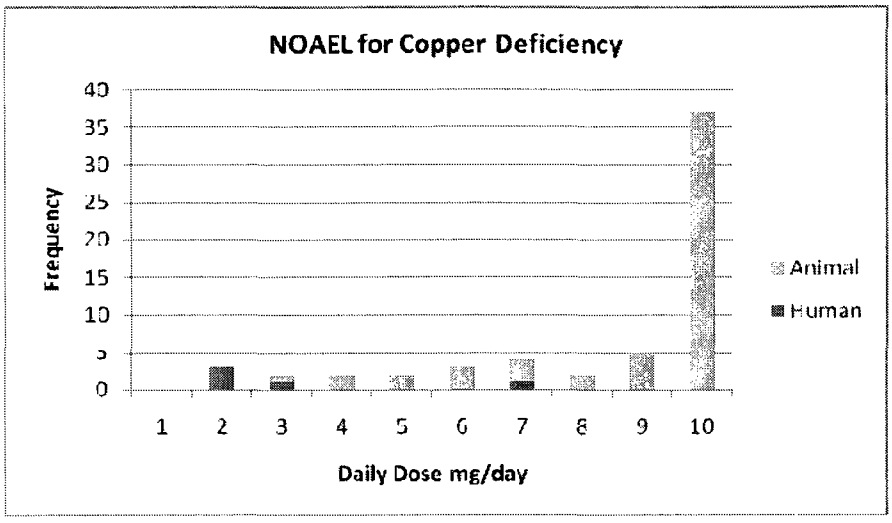


Figure 5.3 NOAELs for Copper Deficiency. Animal Data divided by a factor of 4 and human data multiplied by 1.3.

5.7 Knowledge Translation Strategies for Categorical Regression Results

Renwick et al. (2004), reviewed strategies for the risk-benefit analysis of essential elements, and recommended that risk managers be provided with sufficient information to consider both the risk of deficiency and the risk of excess. They comment on the fact that while the optimum intake level would be where the lines for the exposure-response curves for copper toxicity due to excess and deficiency cross, this would not provide usable health advice for nutritionists or risk managers (Renwick et al., 2004). They recommend that a series of possible lower and upper intake ranges be provided so that the risk manager can select the most appropriate range of intakes, and have information available on the nature of the adverse health effects from the data that was used to form the exposure-response curve (Renwick et al., 2004). Sufficient information should be provided to weigh the relative harm from toxicity due to deficiency and excess (Renwick et al., 2004). Renwick et al. (2004) also

recommend that the data be provided in tabular form to show levels of intake associated with increasing probability of toxicity from deficiency and excess. At this time, categorical regression has not been applied to both the copper excess and deficiency data simultaneously. If we select the point where the two exposure-response curves cross as the optimal intake level, we are implicitly giving equal weight to the risk of copper excess and copper deficiency at severity level 2 or greater.

Table 5.1 lists the responses from human studies in the copper toxicity database that were associated with severity level 2. There are currently more human dietary studies on copper deficiency than copper excess. When estimating an acceptable range of oral intakes, it will be important to evaluate the clinical significance of the responses that have been identified.

Table 5.2 presents the results of the categorical regression analysis in tabular form with the extra risk levels across the top and the severity levels across the side. This table can be used in conjunction with information on the responses that were associated with severity level 2 (Table 5.1) to provide risk managers with sufficient information to set regulatory levels of copper that would prevent adverse effects from both copper excess and deficiency.

Table 5.1: Responses Associated with Severity Level 2 for Copper Deficiency and Excess

Copper Deficiency		*Copper Excess*
Severity Level 2		**Severtity Level 2**
Kelly 1995: Indexes of Immune Response Decreased B lymphocytes, helper lymphocytes (CD4+) and T activated lymphocytes. **Baker et al., 1999b:** Indexes of Bone Resorption Increased pyridinoline Increased deoxypyridinoline **Reiser et al., 1987:** Blood Lipids Increase in plasma LDL Decrease in plasma HDL	← *Homeostatic Range* →	**Pratt 1985:** Decreased Serum Potassium **Turnlund et al. 2004*:** Indexes of Copper Status Increase in Erythrocyte SOD Oxidant Stress Increased Urinary thiobarbituric acid-reactive substances Indexes of Immune Response Change in composition of white blood cells Decreased # of neutrophils Decreased concentration of interleukin-2R (regulations of T cell proliferation)

* Author comments on the fact that the physiologic implications of these changes are unknown.

Table 5.2: ERC-T100 and 90% Two-sided Confidence Intervals (CI) by Severity and Probability of Response, Dose in mg/day – Copper Deficiency and Excess

Severity Level	Copper Deficiency					Copper Excess				
	ERC50	***ERC40***	***ERC30***	***ERC20***	***ERC10***	***ERC10***	***ERC20***	***ERC30***	***ERC40***	***ERC50***
1	1.12 (0.92, 1.36)	1.39 (1.14, 1.69)	1.76 (1.44, 2.15)	2.31 (1.86, 2.86)	3.37 (1.64, 4.30)	1.36 (0.68, 2.38)	2.21 (1.36, 3.91)	3.06 (1.70, 5.27)	4.08 (2.38, 7.13)	5.27 (2.89, 9.17)
2	0.78 (0.63, 0.97)	0.97 (0.78, 1.21)	1.23 (0.98, 1.54)	1.61 (1.27, 2.05)	2.38 (1.79, 3.08)	1.87 (1.19, 3.06)	3.06 (1.87, 4.93)	4.25 (2.55, 6.96)	5.61 (3.40, 9.17)	7.13 (4.25, 12.06)
3	0.41 (0.32, 0.53)	0.51 (0.40, 0.66)	0.65 (0.50, 0.84)	0.85 (0.65, 1.11)	1.24 (0.93, 1.66)	2.21 (1.36, 3.57)	3.57 (2.21, 5.78)	4.93 (3.06, 7.98)	6.62 (4.08, 10.70)	8.32 (5.10, 14.10)
4	0.07 (0.04, 0.10)	0.08 (0.05, 0.13)	0.11 (0.07, 0.16)	0.14 (0.09, 0.21)	0.20 (0.13, 0.31)	4.08 (2.38, 7.13)	6.79 (3.74, 12.23)	9.34 (4.93, 17.66)	12.40 (6.28, 23.95)	15.80 (7.81, 32.10)

Copper deficient data is modeled with the cumulative odds model with the probit link function. Intercept is stratified by the animal species and the concentration parameter is stratified by age. Copper excess data is modeled with the cumulative odds model with the logit link function. The intercept is stratified by the animal species and the route of exposure and the duration parameter is stratified by age.

5.8 Comparison of Results with Average Copper Intakes

While 2.1 mg Cu/day represents the point where the probability response curves for copper excess and deficiency at severity level 2 or higher cross, actual intakes this high are unusual. Data from 10 dietary surveys where dietary copper was assessed by chemical analysis have been pooled to create a frequency distribution (Klevay, 1998; Klevay et al., 1993): only 3.2% of diets exceed 3.0 mg/day, 61% are less than 1.5 mg/day, and 33% are less than 1.0mg/day. Furthermore, the middle quartiles of this curve range from 0.91 to 1.86 mg/day. The diets have been described as representative of those in Belgium, Canada, United Kingdom, and the United States (Klevay et al., 1993c). Klevay (1998) has commented on the fact that the estimates for dietary requirements are likely not too high, but rather, diets are often too low in copper. Klevay (1998) has emphasized that as the Western diet appears to be frequently low in copper in comparison to suggested standards, more attention is needed on essential nutrients including copper in dietary surveys; food and diet analyses; nutrition information; dietary planning; and nutrition research (Klevay, 1998). There is evidence that dietary copper can be increased by avoiding foods low in copper (e.g., foods with high fats and oils) and seeking foods high in copper (e.g., oysters, liver, peanut butter, crab, and legumes). It has also been suggested that the benefits of increasing foods high in copper and decreasing foods low in copper might lower the risk of heart disease (Klevay, 1992; 1993a; 1994). Copper deficiency is the only nutritional imbalance that has been shown to produce hypercholesterolemia, glucose intolerance, abnormal electrocardiograms, hyperuricemia and hypertension in animals (Klevay 1992). Klevay (1992) comments on the fact that while many of the diets analyzed from the 10 food frequency questionnaires were low in copper, some nutritionist may consider those consuming these diets as apparently normal healthy people; however, it is generally agreed that there are many

people in the general population with cholesterol levels that are too high and whose glucose tolerance is too low.

The risk assessment process for copper has traditionally attempted to define the minimum amount of copper that is needed by the body for normal function and the maximum amount that is compatible with normal function (NRC, 1989). Renwick et al. (2004) have provided a useful discussion on the risk-benefit analysis of micronutrients, highlighting the fact that there is a third type of intake-incidence curve if there are additional benefits at intakes above the RDI. There is evidence in the epidemiological literature that intakes above the amount needed to replenish copper stores (approximately 1.5 mg Cu/day) may have some additional health benefits. For example, in Cerhan et al. (2003), any use of supplemental copper was found to decrease the risk of rheumatoid arthritis. Those consuming 2.29 mg Cu/day (1.08 SD) were found to have decreased diastolic blood pressure, circulating glucose, uric acid, and LDL levels, as compared to those consuming 1.12 mg/day (0.29 SD) (Bo et al., 2008). Those who had a copper intake greater than 1.56 mg/day had a 66% reduced risk of lung cancer, compared with those consuming less than 0.99 mg Cu/day (Mahabir et al., 2007).

5.9 Limitations in the Analysis

It is important to recognize the limitations in the categorical regression analysis. In the present meta-analysis of copper toxicity studies using categorical regression, the quality of the final estimates will be influenced by the quality of the individual studies in the copper toxicity database. All studies on rats and mice, and a few studies on humans employed a controlled experimental design. When deciding on which studies were to be included in the copper database, the original group of experts in toxicology and risk assessment not only assessed the utility of each experiment for an exposure-response analysis but also the quality

of the study. Animal studies must have had complete data reporting and employed appropriate statistical analyses. While controlled experimental designs are considered to be the most rigorous of the research design methods, other research design methods were considered due to the limited data available across a wide range of doses and durations of exposure, especially among human studies. For example, two case studies have been included in the copper database, one involving an acute and accidental overdose of copper, and one involving a report of cirrhosis from a chronic exposure to 45 mg Cu/day in supplement form. Case reports involving only a single individual clearly have limited generalizability; however, they are the only studies available showing the potential effects of long-term elevated copper intake or short-term effects of massive copper ingestion in humans. Several studies in the copper database on humans have used a repeated cross-over design. One of the quality considerations for human studies was whether there was a 'washout period' to minimize possible carry-over effects in these types of studies. If a study with a repeated measures design did not have a washout period, only the data from the first experimental period was recorded in the copper toxicity database.

Binning responses into severity categories requires expert judgment in allocating events and endpoints to different categories. This is complicated by the fact that a wide variety of types of data from diverse study designs have used a variety of response measures including counts of subjects having a particular type of response (incidence data) and means and standard deviations of various biochemical measurement in various dose groups (Stern et al., 2007). A number of studies measured a wide range of responses, and reported small but statistically significant differences compared to controls. The challenge is deciding whether statistically significant differences are also clinically significant. Often the physiologic implications of the observed changes are unknown. Guth et al. (1997)

commented on the fact that the assignment of severity scores is more challenging in the categorical regression approach compared to the NOAEL or BMD approaches, as diverse endpoints must be scored consistently across three or more severity levels rather than simply categorizing an endpoint as being adverse or not adverse. There is some uncertainty involved in categorizing responses into six or more severity categories especially when the available studies on copper toxicity span several decades and do not always use standardized technology and terminology.

One of the challenges in working with studies on copper toxicity is that the majority of the information on the adverse effects of copper deficiency and excess is described only at the group level. Assigning group level data to a severity category results in a loss of information about the variability of response but is necessary in order to conduct the categorical regression analysis.

The most interesting studies on chronic copper toxicity are often found in the epidemiological literature, where excess and deficient levels of copper have been associated with several chronic diseases. More research is required to confirm these associations, and to infer causality. There remain challenges in using this data in a categorical regression analysis, as it is difficult to obtain information on the amount of copper consumed daily leading up to the reported adverse effects. The main objective in epidemiological studies where copper is the exposure of interest is to find associations between elevated or deficient copper intake and reduced or increased risk of chronic disease. These studies cannot always be used to inform regulatory standards on exactly how much copper should be consumed. Irrespective of whether these studies can or cannot be used in an exposure-response assessment, they can help in the identification of early biomarkers of copper imbalance. As copper is involved in so many important functions in the body, it can be difficult for

investigators to decide on an appropriate selection of relevant biomarkers to investigate early disruptions of copper imbalance. At this time, the strategy used to assign exposure-response information to severity scores is not suitable for integrating some epidemiological studies. For example, if a study reports that those taking copper supplements have a lower risk of developing kidney cancer, the traditional severity scoring approach would then have to assign the severity score to the negative outcome (kidney cancer); a high severity level would be assigned to the exposure group not taking copper supplements. The central theme of this project is to develop an approach that increases the use of the available evidence when determining regulatory standards. The current approach used to select studies for the copper database and assign the exposure-response information to severity scores for a categorical regression analysis does favour experimental studies. Future initiatives will need to integrate the epidemiological studies.

There are several challenges in the application of categorical regression when there is limited data across different severity levels and ranges of exposure. Model selection options were limited in this analysis due to information gaps in the database that necessitated the use of simpler models and stricter assumptions. There are also several barriers in combining animal and human data including disparate health end points, biological systems, and study designs. A complex model with several levels of stratification may be needed to sufficiently account for the variability in the database.

Categorical regression and other empirical modeling approaches fit generic flexible mathematical models to the data with a limited biological basis. The relationship between exposure and the severity of adverse effects is defined by a mathematical expression where the coefficients for the intercept, concentration, and duration parameter are estimated by statistical analysis of actual data from studies of copper toxicity. In the categorical regression

model that was used to describe the available copper toxicity data, it is assumed that the risk of an adverse event is linearly related to the exposure concentration. It would be ideal to have a model that incorporates information on the biological processes underlying copper toxicity; however, the present model is defined based on statistical characteristics. For example, the choice of the probability function (normal, logistic or Gumbel) does not have a biological basis. Careful consideration is required when deciding how the data will be used in defining the exposure-response model. It is also important to stay within the range of the data when interpreting the results, as extrapolating beyond the data increases the model dependence and models that appear to fit well within the range of the data can have widely different results at doses several orders of magnitudes lower or higher (Haber et al., 2001). While categorical regression fits flexible mathematical models to the data without a biological basis, the use of categorical regression to extrapolate to low or high doses may be more appropriate than BMD modeling, as multiple studies and multiple species are combined in a common analysis.

The CatReg software developed by the US Environmental Protection Agency has been designed specifically for categorical regression analyses of toxicity data taking into account the need for stratification, cluster sampling and interval censoring. The program has considerable potential for becoming a useful tool for conducting categorical regression analyses across a wide range of exposures; however, the software was designed originally for adverse effects from inhalation exposures. As a result, the program only recognizes the concentration and duration variable in the database if it is labeled 'mg/m^3' and 'hours', respectively. As the graphing options are limited, axis labels need to be altered manually to change the concentration and duration labels to their appropriate measures. There are also issues with the visual presentation of the graphs produced from the program. When plotting

all the ERC lines for a defined severity level, it is difficult to interpret which ERC10 lines correspond to which strata. During the course of this work, a few glitches in the program have been identified, including the inability to plot all the observations in the deviance plots when there are more than five strata. As the US EPA is currently updating the CatReg software, several recommendations have been submitted for improving the utility and flexibility of the program.

5.10 Future Research Initiatives

In order to expand the current database for the purpose of improving the categorical regression analysis, there is a need for more studies on humans looking at the effects of marginally excess and deficient levels of copper, and a need for the measurement of a broad range of relevant and sensitive markers of copper toxicity. At this time, there is little information on the long term effects of elevated or deficient copper intake. As there might be a sex difference in susceptibility to copper excess and deficiency, it would be useful for studies to report findings separately for males and females. In order to improve the precision in which dose is characterized within the database, there is a need for more specific information on the bodyweight of the animal species at the start, midpoint and termination of the exposure period, and specific information on daily consumption levels of feed and drinking water. In order to characterize daily dose as milligram per day, milligram per kilogram body weight or an approximation of surface area (e.g., mg/kg $bw^{2/3}$ /day), there is a need for not only information on the amount of copper in feed or drinking water, but also the average amount of feed or water consumed daily. Presently, this information often has to be estimated from standard laboratory guidelines, which can affect the precision with which dose is characterized in the database.

While a categorical regression analysis has been shown to be a useful empirical approach for modeling a diverse collection of studies on copper deficiency and excess, Stern et al. (2007) comment on the fact that: "ideally, detailed information regarding copper uptake, binding, distribution, metabolism and excretion would be coupled with mechanistic models of how various organ systems respond to variation in their copper status." An improved understanding of copper metabolism is needed to derive more precise estimates of dietary requirements.

One important contribution realized during the most recent update of the current copper toxicity database (2008) was the increase in the number of observations on measures of toxicity related to specific organ systems, including the liver. This may ultimately allow for the development of an organ specific exposure-response model. Future initiatives with the copper toxicity database could involve a focused literature review update to identify and incorporate studies that have used subjects with perturbed copper metabolism (e.g., mutant mice) to evaluate differences in risk to excess and deficient levels of copper. The continual update, extraction, and organization of information in the copper toxicity database will be useful in future assessments of copper toxicity.

Copper is not the only essential metallic element where mechanistic models are lacking and where there is insufficient evidence to support a complex exposure-response relationship using traditional empirical approaches. The analytic approach used in this thesis could be replicated for other essential metals. The current models available in CatReg can only define the exposure-response curves for copper deficiency and copper excess separately. There is currently no other statistical technique as advanced as the categorical regression models described by the US EPA that is able to describe a U-shaped exposure-response curve where data on copper deficiency and copper excess is included in a common

analysis. Before developing a statistical technique that can incorporate copper deficiency and excess data in a combined analysis there is a need to consider whether mechanisms of toxicity due to copper deficiency and excess are independent or interrelated at the biological level (Stern et al., 2007).

This preliminary application of categorical regression to model the exposure-response curve for copper is the first attempt to initiate a common risk assessment approach that considers the risk of both copper deficiency and excess. Integrating a broad range of responses of different levels of severity in the categorical regression analysis suggests that current recommendations for copper intake including the RDI (0.9 mg/day) and the tolerable upper intake level (10 mg/day) may not protect the population from responses that might occur outside the limits of the acceptable range of oral intake including increased markers of bone resorption, decreased concentration of immune cells, increased serum potassium, reduced superoxide dismutase activity and altered cholesterol and triglyceride levels in the blood and liver. As categorical regression is able to incorporate a broad range of responses to copper excess and deficiency and allows for the definition of risk at different levels of probability, it offers a way to use more of the available toxicity data when making risk management decisions.

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APPENDIX A: SUMMARY TABLES OF ANIMAL AND HUMAN STUIDES FROM THE COPPER TOXICITY DATABASE

Table A1: References and Observations on Copper Excess

Ref. (ID#)	*Test Type*	*Species*	*Strain*	*Copper Species*	*Route of Admin.*	*Life stage*	*Sex*	*Exp.*	*Grp.*	*Conc.*	*Days*	*Sev.*
Baker 1999a (2)	Subacute Toxicity	Human	NA	Copper sulfate	Capsule	Adult	M	1	1	0 mg/d	42	0
								1	2	3 mg/d	42	0
								1	3	6 mg/d	42	0
							F	2	1	0 mg/d	42	0
								2	2	3 mg/d	42	0
								2	3	6 mg/d	42	0
Pratt 1985 (6)	Subchronic Toxicity	Human	NA	Copper gluconate	Capsule	Adult	B	1	1	0 mg/d	84	0
								1	2	10 mg/d	84	2
Murthy 1981 (10)	Subacute Toxicity	Rats	NS	Copper sulfate pentahydrate	Feed	Adult	M	1	1	0 mg/d	30	0
								1	2	5 mg/d	30	3
Jones 1997 (14)	Subacute	Human	NA	Copper as glycine-chelate	Capsule	NS	M	1	1	0 mg/d	28	0
								1	2	2 mg/d	28	0
Haywood 1985 (20)	Subacute Toxicity	Rats	Wistar	NS	Feed	Weanling	M	1	1	10 mg/kg	7	0
								1	2	3000 mg/kg	7	1
								1	3	4000 mg/kg	7	1
								1	4	5000 mg/kg	7	1
								1	5	6000 mg/kg	7	4
								2	1	10 mg/kg	14	0
								2	2	3000 mg/kg	14	4
								2	3	4000 mg/kg	14	4
								2	4	5000 mg/kg	14	4
								2	5	6000 mg/kg	14	4
								3	1	10 mg/kg	21	0
								3	2	3000 mg/kg	21	4
								3	3	4000 mg/kg	21	4
								3	4	5000 mg/kg	21	4
								3	5	6000 mg/kg	21	4
								4	1	10 mg/kg	28	0
								4	2	3000 mg/kg	28	4

								4	3	4000 mg/kg	28	4
								4	4	5000 mg/kg	28	4
								4	5	6000 mg/kg	28	4
	Subchronic Toxicity							5	1	10 mg/kg	35	0
								5	2	3000 mg/kg	35	4
								5	3	4000 mg/kg	35	4
								5	4	5000 mg/kg	35	4
								5	5	6000 mg/kg	35	5
								6	1	10 mg/kg	42	0
								6	2	3000 mg/kg	42	4
								6	3	4000 mg/kg	42	4
								6	4	5000 mg/kg	42	4
								6	5	6000 mg/kg	42	5
	Chronic Toxicity							7	1	10 mg/kg	105	0
								7	2	3000 mg/kg	105	4
								7	3	4000 mg/kg	105	4
								7	4	5000 mg/kg	105	4
								7	5	6000 mg/kg	105	5
Haywood 1980 (22)	Subacute Toxicity	Rats	NS	Copper sulfate	Feed	Weanling	M	1	1	0 ppm	7	0
								1	2	2000 ppm	7	1
	Subacute Toxicity							2	1	0 ppm	14	0
								2	2	2000 ppm	14	4
	Subacute Toxicity							3	1	0 ppm	21	0
								3	2	2000 ppm	21	4
	Subchronic Toxicity							4	1	0 ppm	42	0
								4	2	2000 ppm	42	4
	Subchronic							5	1	0 ppm	63	0
								5	2	2000 ppm	63	4
	Chronic							6	1	0 ppm	105	0
								6	2	2000 ppm	105	4
Fuentealba 2000 (25)	Chronic	Rats	Fischer 344	Copper sulfate	Feed	Adult	M	1	1	0 ppm	126	0
								1	2	1500 ppm	126	3
							F	2	1	0 ppm	126	0

								2	2	1500 ppm	126	3
Hebert 1993 (26)	Subacute Toxicity	Rats	Fischer 344	Copper sulfate pentahydrate	Water	Adult	M	1	1	0 ppm	15	0
								1	2	300 ppm	15	0
								1	3	1000 ppm	15	0
								1	4	3000 ppm	15	3
								1	5	10000 ppm	15	6
								1	6	30000 ppm	15	6
	Subacute Toxicity	Mice	B6C3F1	Copper sulfate pentahydrate	Water	Adult	M	2	1	0 ppm	15	0
								2	2	300 ppm	15	0
								2	3	1000 ppm	15	0
								2	4	3000 ppm	15	4
								2	5	10000 ppm	15	6
								2	6	30000 ppm	15	6
	Subacute Toxicity	Rats	Fischer 344	Copper sulfate pentahydrate	Water	Adult	F	3	1	0 ppm	15	0
								3	2	300 ppm	15	0
								3	3	1000 ppm	15	0
								3	4	3000 ppm	15	3
								3	5	10000 ppm	15	6
								3	6	30000 ppm	15	6
	Subacute Toxicity	Mice	B6C3F1	Copper sulfate pentahydrate	Water	Adult	F	4	1	0 ppm	15	0
								4	2	300 ppm	15	0
								4	3	1000 ppm	15	3
								4	4	3000 ppm	15	4
								4	5	10000 ppm	15	6
								4	6	30000 ppm	15	6
	Subacute Toxicity	Rats	Fischer 344	Copper sulfate pentahydrate	Feed	Adult	M	5	1	0 ppm	15	0
								5	2	1000 ppm	15	0
								5	3	2000 ppm	15	0
								5	4	4000 ppm	15	0
								5	5	8000 ppm	15	4
								5	6	16000 ppm	15	4
	Subacute Toxicity	Rats	Fischer 344	Copper sulfate pentahydrate	Feed	Adult	F	6	1	0 ppm	15	0
								6	2	1000 ppm	15	0

							6	3	2000 ppm	15	0
							6	4	4000 ppm	15	3
							6	5	8000 ppm	15	4
							6	6	16000 ppm	15	4
Subacute Toxicity	Mice	B6C3F1	Copper sulfate pentahydrate	Feed	Adult	M	7	1	0 ppm	15	0
							7	2	1000 ppm	15	0
							7	3	2000 ppm	15	0
							7	4	4000 ppm	15	4
							7	5	8000 ppm	15	4
							7	6	16000 ppm	15	4
Subacute Toxicity	Mice	B6C3F1	Copper sulfate pentahydrate	Feed	Adult	F	8	1	0 ppm	15	0
							8	2	1000 ppm	15	0
							8	3	2000 ppm	15	0
							8	4	4000 ppm	15	4
							8	5	8000 ppm	15	4
							8	6	16000 ppm	15	4
Subchronic Toxicity	Rats	Fischer 344	Copper sulfate pentahydrate	Feed	Adult	M	9	1	0 ppm	92	0
							9	2	500 ppm	92	1
							9	3	1000 ppm	92	3
							9	4	2000 ppm	92	4
							9	5	4000 ppm	92	4
							9	6	8000 ppm	92	4
Subchronic Toxicity	Rats	Fischer 344	Copper sulfate pentahydrate	Feed	Adult	F	10	1	0 ppm	92	0
							10	2	500 ppm	92	1
							10	3	1000 ppm	92	3
							10	4	2000 ppm	92	4
							10	5	4000 ppm	92	4
							10	6	8000 ppm	92	4
Chronic Toxicity	Mice	B6C3F1	Copper sulfate pentahydrate	Feed	Adult	M	11	1	0 ppm	92	0
							11	2	1000 ppm	92	0
							11	3	2000 ppm	92	0
							11	4	4000 ppm	92	4
							11	5	8000 ppm	92	4

								11	6	16000 ppm	92	4
	Chronic Toxicity	Mice	B6C3F1	Copper sulfate pentahydrate	Feed	Adult	F	12	1	0 ppm	92	0
								12	2	1000 ppm	92	0
								12	3	2000 ppm	92	0
								12	4	4000 ppm	92	4
								12	5	8000 ppm	92	4
								12	6	16000 ppm	92	4
Araya 2001 (35)	Acute Toxicity	Human	NA	Copper sulfate pentahydrate	Water	Adult	B	1	1	0 mg/L	1	0
								1	2	2 mg/L	1	0
								1	3	4 mg/L	1	0
								1	4	6 mg/L	1	4
								1	5	8 mg/L	1	4
Baker 1999b (37)	Subacute Toxicity	Humans	NA	Copper sulfate pentahydrate	Diet	Adult	M	1	1	1.6 mg/day	42	0
										6.0 mg/day	42	0
Cristofori 1992 (42)	Chronic Toxicity	Rats	Spraque-Dawley	NS	Diet	Adult	F	1	1	5 ppm	210	0
								1	2	200 ppm	210	2
	Chronic Toxicity	Rats	Spraque-Dawley	NS	Diet	Adult	F	2	1	5 ppm	350	0
								2	2	200 ppm	350	2
Cromwell 1989 (43)	Subacute Toxicity	Pig	Hamshire-Yorkshire	Copper sulfate pentahydrate	Diet	Weanling	B	1	1	0 ppm	28	0
								1	2	125 ppm	28	3
								1	3	250 ppm	28	3
				Copper Oxide				2	1	0 ppm	28	0
								2	2	125 ppm	28	0
								2	3	250 ppm	28	0
Cunnane 1985 (44)	Subchronic Toxicity	Rats	Sprague-Dawley	NS	Diet	Weaning	M	1	1	6 mg/kg/d	84	0
								1	2	250 mg/kg/d	84	3
Fuentealba 1989 (48)	Subacute Toxicity	Rats	Wistar	NS	Diet	Weaning	M	1	1	20 ppm	7	0
								1	2	1500 ppm	7	4
								2	1	20 ppm	28	0
								2	2	1500 ppm	28	4
	Subchronic Toxicity							3	1	20 ppm	56	0
								3	2	1500 ppm	56	4
								4	1	20 ppm	84	0

								4	2	1500 ppm	84	4
	Chronic Toxicity							5	1	20 ppm	112	0
								5	2	1500 ppm	112	4
Giovanetti 1998 (50)	Subacute Toxicity	Mice	B6C3F1	Copper sulfate pentahydrate	Diet	Weaning	M	1	1	4.98 ppm	28	0
								1	2	200 ppm	28	3
Gotteland 2001 (54)	Acute Toxicity	Humans	NS	Copper sulfate pentahydrate	Water	Adults	B	1	1	10 mg/L	1	4
Greene 1987 (55)	Chronic Toxicity	Rats	Sprague-Dawley	NS	Diet	Weaning	M	1	1	25 ppm	112	0
										100 ppm	112	0
Gross 1989 (56)	Chronic Toxicity	Rats	Sprague-Dawley	Copper acetate	Water	Adult	M	1	1	0 %	252	0
								1	2	0.0125 %	252	3
Jantsch 1985(63)	Acute Toxicity	Human	NA	Copper sulfate pentahydrate	Diet	Adult	M	1	1	250 g/day	1	4
Liu 1986 (75)	Chronic Toxicity	Rats	Wistar	Copper carbonate	Diet	Weaning	M	1	1	18 mg/kg/d	105	0
								1	2	100 mg/kg/d	105	3
Mullins 1998 (80)	Subchronic Toxicity	Rats	Wistar	Copper sulfate pentahydrate	Diet	Adults	M	1	1	10 mg/kg	42	0
								1	2	1500 mg/kg	42	2
O'Donohue 1999 (82)	Chronic Toxicity	Humans	NS	NS	Capsule	Adults	M	1	1	45 mg/day	1095	4
Zhang 2000 (98)	Subchronic Toxicity	Rats	Wistar	Copper sulfate pentahydrate	Capsule	Adult	B	1	1	0 mg/kg/d	40	0
								1	2	500 mg/kg/d	40	3
Rana 1980 (99)	Subacute	Rats	NS	Copper sulfate pentahydrate	Diet	Adult	M	1	1	0 mg/kg/day	20	0
								1	2	100 mg/kg/day	20	4
Massie 1984 (103)	Chronic Toxicity	Mice	C57B1/6J	Cu gluconate	Water	Adult	M	1	1	0 ppm	NA[a]	0
								1	2	317 ppm	NA[a]	6
Pizarro 1999b (104)	Subchronic Toxicity	Human	NA	Copper sulfate pentahydrate	Water	Adult	F	1	1	0 mg/L	77	0
								1	2	1 mg/L	77	0
								1	3	3 mg/L	77	4
								1	4	5 mg/L	77	4
Araya 2003a (109)	Acute Toxicity	Humans	NA	Copper sulfate pentahydrate	Water	Adult	B	1	1	0 mg/l	1	0
								1	2	10 mg/l	1	4
Araya 2003b (110)	Acute Toxicity	Humans	NA	Copper sulfate pentahydrate	Water	Adult	F	1	1	0 mg/l	1	0
								1	2	2 mg/l	1	0
								1	3	4 mg/l	1	0
								1	4	6 mg/l	1	4
								1	5	8 mg/l	1	4

Araya 2003c (111)	Subchronic Toxicity	Humans	NA	Copper sulfate pentahydrate	Water	Adult	B	1	1	0.01 mg/l	60	0
								1	2	2 mg/l	60	0
								1	3	4 mg/l	60	0
								1	4	6 mg/l	60	4
Araya 2004 (112)	Subchronic Toxicity	Humans	NA	Copper sulfate pentahydrate	Water	Adult	B	1	1	0 mg/l	60	0
								1	2	2 mg/l	60	0
								1	3	4 mg/l	60	4
								1	4	5 mg/l	60	4
Armstrong 2004 (114)	Subchronic	Pigs	NS	Copper sulfate pentahydrate	Feed	Weanling	B	1	1	10 ppm	40	0
								1	2	135 ppm	40	3
								1	3	260 ppm	40	3
				Copper citrate				2	1	15 ppm	40	0
								2	2	46 ppm	40	0
								2	3	77 ppm	40	0
								2	4	140 ppm	40	3
Cisternas 2005 (117)	Chronic	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Feed	Weanling	B	1	1	10 ppm	120	0
								1	2	1200ppm	120	4
Feng 2007 (126)	Subacute	Pigs	NS	Copper sulfate pentahydrate	Feed	NS	B	1	1	12.4 mg/kg	30	0
								1	2	250 mg/kg	30	2
								2	1	12.4 mg/kg	30	0
				Copper Proteinate				2	2	50 mg/kg	30	0
								2	3	100 mg/kg	30	2
Kvietkauskaite 2004 (136)	Subchronic	Mice	BALB/c	Copper sulfate pentahydrate	Water	Adults	M	1	1	0 mg/kg bw/d	133	0
								1	2	22 mg/kg bw/d	133	3
								1	3	42 mg/kg bw/d	133	3
O'Connor 2003 (138)	Subacute	Humans	NA	Copper sulfate pentahydrate	Capsule	Adult	B	1	1	1.23 mg/d	42	0
								1	2	4.23 mg/d	42	1
				Copper glycine chelates				2	1	1.23 mg/d	42	0
								2	2	4.23 mg/d	42	1
								3	1	1.23 mg/d	42	0
								3	2	7.23 mg/d	42	1
Ozcelik 2002 (140)	Subchronic	Rats	Wistar albino	Copper sulfate pentahydrate	Water	Adult	B	1	1	0 µg/mL	54	0
								1	2	250 µg/mL	54	3

Turnlund 2004 (146)	Subacute	Humans	NA	NS	Supplement	Adult	M	1	1	7.8 mg/d	18	2
Alissa 2004 (152)	Subchronic	Rabbits	New Zealand White	NS	Feed	Adult	M	1	1	3.7 mg/d	84	0
								1	2	350 mg/d	84	3
Becaria 2006 (158)	Subchronic	Mice	B6C3F1	Copper sulfate pentahydrate	Water	Adult	M	1	1	0 ppm	84	0
								1	2	2 ppm	84	3
Davis 2002 (172)	Subacute	Pigs	NS	NS	Feed	Weanling	B	1	1	20 ppm	10	0
								1	2	195 ppm	10	3
Goldschmith 2005 (178)	Subacute	Rats	NS	Copper sulfate pentahydrate	Water	Adult	B	1	1	0.12 mg/d	20	0
								1	2	12.12 mg/d	20	4
Gurel 2007 (180)	Subchronic	Rats	Sprague-Dawley	NS	Water	Adult	F	1	1	0 mg/l	60	0
								1	2	100 mg/l	60	4
								1	3	400 mg/l	60	4
Lai 2005 (187)	Subacute	Rats	Wistar	Copper sulfate pentahydrate	Water	Weanling	M	1	1	0 μg/mL	7	0
								1	2	50 μg/mL	7	0
								1	3	100 μg/mL	7	0
								1	4	200 μg/mL	7	3

[a]Exposure duration = lifespan of each subject 500-975.

Note. Ref. (ID#), reference and identification number; M, male; F, female; B, male and female; Exp., experiment number within the publication; Grp., group number within the experiment; Conc., concentration reported in the study; Sev., severity score assigned.

Table A2: References and Observations on Copper Deficiency

Ref (ID#)	*Exposure Duration Categories*	*Species*	*Strain*	*Copper Species*	*Route of Admin.*	*Life stage*	*Sex*	*Exp.*	*Grp.*	*Conc.*	*Days*	Sev
Arce 1992 (1)	Subchronic	Mice	Swiss Webster	Copper Sulfate	Feed	Adult	F	1	1	1 ppm	39	2
								1	2	10 ppm	39	0
DiSilvestro 1992 (4)	Subchronic	Rats	Sprague-Dawley	Copper Sulfate	Feed	Postweanling	M	1	1	8 ppm	42	0
								1	2	2.5 ppm	42	2
								1	3	0.2 ppm	42	3
Klevay 1985 (8)	Chronic	Mice	Swiss Webster	Copper sulfate pentahydrate	Water	Adult	F	1	1	0 µg/ml	NA[a]	3
								1	2	10 µg/ml	NA[a]	0
Schuschke 1999 (16)	Subacute	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Feed	Weaning	M	1	1	0 ppm	28	3
								1	2	1.5 ppm	28	3
								1	3	3 ppm	28	3
								1	4	6 ppm	28	0
Schuschke 1995 (17)	Subacute	Rats	Sprague-Dawley	NS	Feed	Weaning	M	1	1	6 ppm	7	0
								1	2	3 ppm	7	1
								1	3	1.5 ppm	7	3
								2	1	1.5 ppm	21	3
								2	2	3 ppm	21	1
								2	3	6 ppm	21	0
								3	1	1.5 ppm	35	3
								3	2	3 ppm	35	3
								3	3	6 ppm	35	0
Kelley 1995 (18)	Subacute	Human	NA	NS	Diet	Young adults	M	1	1	0.66 mg/d	24	2
Prohaska 1995 (19)	Subchronic	Rats	Sprague-Dawley	Copper sulfate	Water	Weanling	M	1	1	0 mg/L	38.5	3
								1	2	20 mg/L	38.5	0
Saari 1999 (24)	Subchronic	Rats	Sprague-Dawley	NS	Feed	Weanling	M	1	1	6 ppm	35	0
								1	2	0.8 ppm	35	3
								1	3	0.4 ppm	35	3
								1	4	0 ppm	35	3
Menino 1986 (27)	Subchronic	Mice	Swiss-Webster	Copper carbonate	Feed	Adult	F	1	1	11 ppm	60	0
								1	2	6 ppm	60	0

								1	3	5 ppm	60	0
								1	4	4 ppm	60	0
								1	5	3 ppm	60	3
								1	6	2 ppm	60	4
								1	7	1 ppm	60	4
Turnlund 1990 (31)	Subchronic	Humans	NA	Copper sulfate pentahydrate	Diet	Adult	M	1	1	0.785 mg/d	90	1
								1	2	1.68 mg/d	90	0
Allen 1996 (32)	Chronic	Rats	Sprague-Dawley	Copper carbonate	Diet	Weaning	M	1	1	5.79 mg/kg	140	0
								1	2	0.46 mg/kg	140	3
Allen 1978 (33)	Subchronic	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Diet	Weaning	M	1	1	0.57 µg	63	3
								1	2	5 µg	63	0
Allen 1988 (34)	Subchronic	Rats	Rowett	NS	Diet	Weaning	M	1	1	0.2 µg	49	2
								1	2	10 µg	49	0
Baker 1999b (37)	Subchronic	Human	NA	Copper sulfate pentahydrate	Diet	Adult	M	1	1	1.6 mg/d	42	0
								1	2	0.7 mg/d	42	2
Bala 1990 (38)	Subchronic	Rats	Lewis	Copper carbonate	Diet	Weaned	M	1	1	0.6 µg/g	35	3
								1	2	6 µg/g	35	0
						Birth-5weeks post weanling	M	2	1	0.6 µg/g	56	2
								2	2	6 µg/g	56	0
Bala 1992 (39)	Subchronic	Pig	NS	NS	Diet	Weanling	B	1	1	0.8 mg/kg/d	77	3
								1	2	6.4 mg/kg/d	77	0
Bode 1992 (40)	Subacute	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Diet	Weanling	M	1	1	0.4 g/kg	28	3
								1	2	5.2 g/kg	28	0
Bremner 1987 (41)	Subchronic	Rats	Hooded Lister	Copper sulfate pentahydrate	Diet	Post-weanling	M	1	1	0.15 mg/kg/d	42	3
								1	2	10 mg/kg/d	42	0
Cunnane 1985 (44)	Subchronic	Rats	Sprague-Dawley	NS	Diet	Weaning	M	1	1	1 mg/kg/d	84	3
								1	2	6 mg/kg/d	84	0
Davidson 1992 (45)	Subchronic	Rats	Sprague-Dawley	Copper carbonate	Diet	Weanling	M	1	1	6.2 µmol/kg	35	3
								1	2	92.4 µmol/kg	35	0
Fields 1997 (47)	Subacute	Rats	Sprague-Dawley	NS	Diet	Weanling	B	1	1	0.6 µg/g	28	3
								1	2	6 µg/g	28	0
Giovanetti 1998 (50)	Subacute	Mice	B6C3F1	Copper sulfate pentahydrate	Diet	Weanling	M	1	1	0.44 ppm	28	3
								1	2	4.98 ppm	28	0

Gitlin 1992 (51)	Subacute	Rats	Sprague-Dawley	NS	Diet	Adult	B	1	1	0.6 mg/kg/d	28	2
								1	2	6 mg/kg/d	28	0
Gomi 1995 (52)	Subchronic	Rats	Fischer 344	Copper oxide	Diet	Adult	F	1	1	0.4 mg/kg/d	70	2
								1	2	5.7 mg/kg/d	70	0
						Adult		2	1	0.4 mg/kg/d	70	3
								2	2	5.7 mg/kg/d	70	0
Goodman 1970 (53)	Subchronic	Rats	Wistar	Copper sulfate pentahydrate	Water	Weanling	M	1	1	0 mg/L	60	3
								1	2	40 mg/L	60	0
Greene 1987 (55)	Chronic	Rats	Sprague-Dawley	NS	Diet	Weanling	M	1	1	0.6 ppm	112	4
								1	2	25 ppm	112	0
Hamilton 2000 (58)	Subchronic	Mice	C57846	NS	Diet	Weaning	M	1	1	0.6 mg/kg/d	98	4
								1	2	2 mg/kg/d	98	4
								1	3	6 mg/kg/d	98	0
Hopkins 1995 (62)	Chronic	Rats	Sprague-Dawley	Copper carbonate	Diet	Weanling	B	1	1	2.8 mg/kg/d	161	3
								1	2	6.6 mg/kg/d	161	0
Johnson 1993 (66)	Subchronic	Rats	Sprague-Dawley	NS	Diet	Weanling	M	1	1	0.2 µg/g	35	3
								1	2	1 µg/g	35	3
								1	3	2 µg/g	35	2
								1	4	3 µg/g	35	2
								1	5	4 µg/g	35	0
Kang 2000 (67)	Subchronic	Mice	FVB	NS	Diet	Weanling	B	1	1	0.35 mg/kg/d	35	3
								1	2	6 mg/kg/d	35	0
Karimbakas 1998 (68)	Subacute	Mice	ICR	NS	Diet	Weanling	M	1	1	1.05 µg/g	21	3
								1	2	6.4 µg/g	21	0
Klevay 1981 (70)	Subchronic	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Diet	Weanling	M	1	1	0.79 µg/g	35	6
								1	2	3.79 µg/g	35	0
Klevay 1986 (71)	Subchronic	Humans	NA	NS	Diet	Adults	M	1	1	0.78 mg/d	150	3
Lai 1995 (72)	Subacute	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Water	Weanling	M	1	1	0 µg/ml	28	3
								1	2	3 µg/ml	28	0
Lai 1994 (73)	Subacute	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Water	Weanling	M	1	1	0 µg/ml	28	2
								1	2	3 µg/ml	28	0
Lai 1996 (74)	Subacute	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Water	Weanling	M	1	1	0 µg/ml	28	3
								1	2	3 µg/ml	28	0

Lynch 1994 (76)	Subchronic	Mice	Swiss-Webster	NS	Diet	Adults	B	1	1	0.3 mg/kg/d	49	3
								1	2	8.4 mg/kg/d	49	0
Mao 1998 (77)	Subchronic	Rats	Sprague-Dawley	NS	Diet	Weanling	M	1	1	1 mg/kg/d	77	3
								1	2	7 mg/kg/d	77	0
Mao 1999 (78)	Subchronic	Rats	Long-Evans	Copper carbonate	Diet	Weanling	M	1	1	2.7 mg/kg/d	84	3
								1	2	6.2 mg/kg/d	84	0
Nelson 1992 (81)	Subacute	Rats	Sprague-Dawley	NS	Feed	Weanling	M	1	1	0.8 mg/kg/d	42	3
								1	2	1.7 mg/kg/d	42	3
								1	3	6.7 mg/kg/d	42	0
Olin 1994 (83)	Subacute	Rats	Sprague-Dawley	NS	Diet	Weanling	B	1	1	7.9 nmol/g	21	3
								1	2	125.9 nmol/g	21	0
Prohaska 2001 (84)	Subacute	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Water	Weaning	F	1	1	20 mg/L/d	30	0
								1	2	0 mg/L/d	30	3
Prohaska 1982 (85)	Subchronic	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Water	Weanling	M	1	1	0 ppm	35	3
								1	2	20 ppm	35	0
Prohaska 1994 (86)	Subacute	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Diet	Infant	B	1	1	0.4 mg/kg/day	28	3
								1	2	4 mg/kg/day	28	0
Rock 1995 (89)	Subchronic	Rats	Wistar	Copper carbonate	Diet	Weanling	M	1	1	0.6 mg/kg/d	42	3
								1	2	7.5 mg/kg/d	42	0
Saari 2002a (90)	Subchronic	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Diet	Weanling	M	1	1	0.27 mg/kg/d	35	3
								1	2	1.43 mg/kg/d	35	2
								1	3	2.92 mg/kg/d	35	2
								1	4	4.27 mg/kg/d	35	0
								1	5	6.15 mg/kg/d	35	0
Saari 2002b (91)	Subchronic	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Diet	Weanling	M	1	1	0 mg/kg/d	35	3
								1	2	1.6 mg/kg/d	35	1
								1	3	3.2 mg/kg/d	35	1
								1	4	24 mg/kg/d	35	0
Sugawara 1999 (92)	Subchronic	Rats	Long-Evans	Copper chloride	Diet	Adults	B	1	1	0.5 mg/kg/d	35	2
								1	2	10 mg/kg/d	35	0
Wang 1996 (95)	Subchronic	Rats	Sprague-Dawley	Copper carbonate	Diet	Weaning	M	1	1	9.4 μmol/kg	42	3
								1	2	103.9 μmol/kg	42	0
Wildman 1995 (96)	Chronic	Rats	Sprague-Dawley	NS	Diet	Weaning	M	1	1	1.3 mg/kg/d	154	3

								1	2	2.8 mg/kg/d	154	3
								1	3	6.7 mg/kg/d	154	0
Rayssiguier 1993 (100)	Subchronic	Rats	Wistar	Copper carbonate	Diet	Weaning	M	1	1	0.6 mg/kg/d	42	3
								1	2	7.5 mg/kg/d	42	0
Reiser 1987 (102)	Subchronic	Humans	NA	NS	Diet	Adult	M	1	1	0.36mg/1000kcal	98	2
								1	2	0.57mg/1000 kcal	98	0
Allen 1978 (106)	Chronic	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Diet	Weanling	M	1	1	0.57 µg/g	168	2
								1	2	5 µg/g	168	0
Ajayi 2005 (107)	Subchronic	Rats	White Albino	Copper carbonate	Diet	Weanling	M	1	1	0.06 mg/kg	42	3
								1	2	20.03 mg/kg	42	0
Andersen 2007 (108)	Subchronic	Rats	Rowett Lister	Copper sulfate pentahydrate	Diet	Weanling	F	1	1	5 mg/kg	49	0
								1	2	2.5 mg/kg	49	3
								1	3	0.75 mg/kg	49	3
Auclair 2006 (115)	Subchronic Toxicity	Mice	C57BL6	Cupric carbonate	Feed	Adults	M	1	1	6 ppm	84	0
								1	2	0.5 ppm	84	3
Cockell 2002 (118)	Subacute Toxicity	Rats	Long Evans	NS	Feed	Weanling	M	1	1	6.19 mg/kg	28	0
								1	2	0.43 mg/kg	28	3
Cockell 2005 (119)	Subacute Toxicity	Rats	Long Evans	NS	Feed	Weanling	M	1	1	6 mg/kg	30	0
								1	2	0.5 mg/kg	30	3
Lucca 2002 (120)	Subacute Toxicity	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Feed	Weanling	M	1	1	5.6 mg/kg	30	0
								1	2	0.66 mg/kg	30	4
Davis 2002 (121)	Subacute Toxicity	Rats	Fisher 344	NS	Feed	Weanling	M	1	1	5.3 µg/g	28	0
								1	2	0.8 µg/g	28	2
Davis 2003 (122)	Subacute Toxicity	Humans	NS	Copper sulfate pentahydrate	Diet	Adults	M	1	1	2.59 mg/d	42	0
								1	2	0.59 mg/d	42	3
Dong 2005 (123)	Subchronic toxicity	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Feed	Weanling	M	1	1	6 mg/kg	35	0
								1	2	0.5 mg/kg	35	4
Falcone 2005 (125)	Chronic Toxicity	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Feed	Adult	M	1	1	5.88 mg/kg	180	0
								1	2	2.94 mg/kg	180	0
								1	3	1.62 mg/kg	180	1
Harvey 2003 (129)	Subacute Toxicity	Humans	NA	Copper chloride	Diet	Adult	M	1	1	6.0 mg/d	56	0
								1	2	1.6 mg/d	56	1
								1	3	0.7 mg/d	56	1

Johnson 2005 (133)	Subchronic Toxicity	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Feed	Weanling	M	1	1	6 mg/kg	42	0
								1	2	3 mg/kg	42	1
								1	3	2.5 mg/kg	42	1
								1	4	2 mg/kg	42	1
								1	5	1.5 mg/kg	42	2
								1	6	1 mg/kg	42	2
								1	7	0.63 mg/kg	42	3
							F	2	1	6 mg/kg	42	0
								2	2	3 mg/kg	42	0
								2	3	2.5 mg/kg	42	1
								2	4	2 mg/kg	42	1
								2	5	1.5 mg/kg	42	2
								2	6	1 mg/kg	42	2
								2	7	0.63 mg/kg	42	3
Li 2005 (137)	Chronic	Rats	Sprague-Dawley	NS	Feed	Adults	M	1	1	5.7 mg/kg	470	0
								1	2	3.1 mg/kg	470	4
								1	3	1.65 mg/kg	470	4
Prohaska 2003 (141)	Subchronic	Mice	Swiss Webster	Copper sulfate pentahydrate	Feed	Adult	F	1	1	20 mg/L	35	0
								1	2	0 mg/L	35	3
		Rats	Holtzman			Adult	F	2	1	20 mg/L	35	0
								2	2	0 mg/L	35	2
Saari 2002 (142)	Subacute	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Feed	Weanling	M	1	1	7.28 mg/kg	35	0
								1	2	2.45 mg/kg	35	3
								1	3	0.79 mg/kg	35	3
								1	4	0.37 mg/kg	35	3
Saari 2007 (143)	Subchronic	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Feed	Weanling	M	1	1	6 mg/kg	35	0
								1	2	0.3 mg/kg	35	3
Schuschke 2002 (144)	Subacute	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Feed	Weanling	M	1	1	6.18 mg/kg	28	0
								1	2	0.29 mg/kg	28	3
Welch 2007 (148)	Subchronic	Rats	Sprague-Dawley	NS	Feed	Weanling	M	1	1	10.5 mg/kg	60	0
								1	2	0.43 mg/kg	60	3
Zeng 2007 (149)	Subchronic	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Feed	Weanling	M	1	1	6.26 mg/kg	35	0
								1	2	0.16 mg/kg	35	3

Chen 2002 (167)	Subchronic	Rats	Long Evans	NS	Feed	Weanling	M	1	1	7.19 mg/kg	35	0
								1	2	0.78 mg/kg	35	3
Gobejishvili 2002 (177)	Subacute	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Feed	Weanling	M	1	1	5.6 mg/kg	28	0
								1	2	0.33 mg/kg	28	4
Gordon 2005 (179)	Subacute	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Feed	Weanling	M	1	1	6.18 mg/kg	28	0
								1	2	0.29 mg/kg	28	3
Johnson 2004 (183)	Subchronic	Rats	Sprague-Dawley	Copper sulfate pentahydrate	Feed	Weanling	M	1	1	5.4 mg/kg	35	0
								1	2	0.3 mg/kg	35	3
Klaahsen 2007 (185)	Subacute	Rats	Long Evans	Cupric carbonate	Feed	Weanling	M	1	1	6 mg/kg	35	0
								1	2	0 mg/kg	35	3
Reeves 2005 (202)	Subacute	Rats	Sprague-Dawley	NS	Feed	Weanling	M	1	1	5.0 mg/kg	19	0
								1	2	0.25 mg/kg	19	3
							F	2	1	5.0 mg/kg	19	0
								2	2	0.25 mg/kg	19	3
Smith 2002 (211)	Subchronic	Rats	Sprague-Dawley	NS	Feed	Adult	M	1	1	5.7 mg/kg	49	0
								1	2	1.1 mg/kg	49	3

[a]Exposure duration = lifespan of each subject 500-975.

Note. Ref. (ID#), reference and identification number; M, male; F, female; B, male and female; Exp., experiment number within the publication; Grp., group number within the experiment; Conc., concentration reported in the study; Sev., severity score assigned.

APPENDIX B: LINK FUNCTION OPTIONS IN CATREG

The following description and examples around the link function options in CatReg was adapted from the United States Environmental Protection Agency's user manual (2000) which provides both a technical and theoretical background on the procedures used to conduct the categorical regression analysis.

Equation B1 describes the cumulative odds model in CatReg:

$$Pr(Y \geq s|C,T) = H[\alpha_s + \beta_1 * f_1(C) + \beta_2 * f_2(T)] \qquad \text{Equation B1}$$

Three forms of H are currently supported by the program including:

Logistic Probability Function: $$H(x) = \frac{e^x}{1+e^x}$$

Normal Probability Function: $$H(x) = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{x} e^{-\frac{z^2}{2}}\,dz$$

Gumbel Probability Function: $$H(x) = 1 - \exp(-e^x)$$

The corresponding link functions (L) (inverse of H) correspond to the probability functions described below.

Logit Link Function: $$L(p) = \log\left[\frac{p}{1-p}\right],$$

Probit Link Function: $$L(p) = 100\ pth\ percentile\ of\ normal\ (0,1), and$$

C log-log Link Function: $$L(p) = \log[-\log(1-p)],$$

Probability (p) corresponds to any number between 0 and 1. The link function and probability function are inverse to each other: H[(L(p)] = p and L[H(x)]=x.

Equation 2 below applies the link function to both sides of model 1 to demonstrate that the link-transformed probability follows a linear model.

$L[\Pr(Y \geq s|C,T)] = \alpha_s + \beta_1 * f_1(C) + \beta_2 * f_2(T),$ Equation B2

s = 1, 2, ..., S.

The purpose of the link function is to ensure that the liner model does not become unbounded leading to meaningless estimates of probabilities for extreme values of C and T (e.g. negative probabilities or probabilities greater than 1).

Link functions can be derived from a basic assumption that the ordinal severity scores correspond to exceeding an underlying toxic response threshold. Under this approach, the ordinal response score is considered a quantal response as it is a quantization of the underlying response (US EPA, 2000; Morgan, 1992). When the coefficients for concentration and duration are constant across severity categories (cumulative odds model) data from one severity category will add information to the modeling of another category.

The CatReg user manual provides a toxicological interpretation of the link function. Let Z represent a particular measure of health for a randomly selected subject, given exposure to concentration C for duration T. Larger values for Z correspond with a healthier individual. If we assume that the health variable Z is distributed in the population as:

$$\Pr(Z \leq x) = H\left(\frac{x-\mu}{\sigma}\right)$$

Where x is equal to a possible value for an individual's health, μ determines the median health for the given level of exposure (C and T), and σ is a measure of the population variation in health.

If H is Gaussian, then the health levels in the population are distributed normally with mean μ and standard deviation σ. As μ increases, the healthier the population is at the given level of exposure (C, T). To model of the situation when health deteriorates as exposure increases, one could suppose that:

$$\frac{\mu}{\sigma} = -\beta_1 * f_1(C) - \beta_2 * f_2(T).$$

We could further assume that a toxic reaction of severity Y≥s occurs if the health measure Z is below a threshold $\sigma\alpha_s$, where σ is specific to the health measure. Under exposure (C, T), the probability of toxic severity of category s or higher is:

$$\Pr(Y \geq s) = \Pr(Z \leq \sigma\alpha_s) = H[\alpha_s + \beta_1 * f_1(C) + \beta_2 * f_2(T)]$$

This equation also corresponds with the cumulative odds model (Equation B1). The link function is a reflection of the underlying distribution of Z, which cannot be measured directly, but its distribution, in particular the dependence on C and T, can be estimated indirectly from toxicological response data (US EPA, 2000).

APPENDIX C: STRATIFICATION OPTIONS IN CATREG

Stratification is used to allow systematically different subsets of the data to have different values for some or all of the parameters. The CatReg user manual (2000) describes an example where stratification options may be relevant. In this example the exposure-response data include mortality results from experiments using rats and mice. The basic explanatory variables are atmospheric toxicant concentration and duration of exposure. The response score equals 0 for surviving animals and 1 for animals that died. In this case, the maximum severity score is S=1. Assuming C and T enter the model logarithmically, the basic model is defined in Equation C1.

Equation C1:

$$L[\Pr(Y = 1|C,T)] = \alpha_1 1 + \beta_1 1 * \log_1 10 [(kC) + \beta_1 2 * \log_1 10(T).]$$

As there are different rates of respiration and metabolism among rats and mice, it may be reasonable to assume that the internal dose for rats should be rescaled compared to that of mice. We could assume that a concentration of C for rats is equivalent to a concentration kC for mice, where k is common to all mice in the study. Then for mice, the model would be defined by Equation C2.

Equation C2:

$$L[\Pr(Y = 1|C,T)] = \alpha_1 1 + \beta_1 1 * \log_1 10(kC) + \beta_1 2 * \log_1 10\ (T)$$
$$= [\alpha_1 + \beta_1 * \log_{10}(k)] + \beta_1 * \log_{10}(C) + \beta_2 * \log_{10}(T)$$

Equation C2 demonstrates that mice (MU) have a different intercept than rats (RT), where $\alpha_1^{MU} = \alpha_1^{RT} + \beta_1 * \log_{10}(k)$. By stratifying the intercept parameter, the data are allowed to determine the estimate of the conversion factor k. CatReg provides the ability to stratify the intercept, concentration parameter and/or the duration parameter.

APPENDIX D: GENERALIZED LIKELIHOOD ESTIMATION

The following information on generalized likelihood estimation is from the USA Environmental Protection Agency's manual on CatReg (2000). The CatReg user manual comments on the fact that the weighed ordinal regression analysis corresponds to a modified likelihood in which the probability associated with the ith observation is raised to a power of w_i. The results in a modified likelihood with a weighted deviance:

$$Deviance = -2\sum_{i=1}^{N} w_i \log[P_i(L_i) - P_i(U_i + 1)]$$

When the weights do not correspond to incidences, then this likelihood corresponds to a nonstandard ordinal regression model. With the nonstandard ordinal regression model, it is more common to interpret the deviance as a generalized criterion and to assume that the usual original regression model holds. Under this assumption, the generalized deviance still leads to consistent estimates of the parameters, but it does not correspond to the likelihood of the data. The estimator is defined by a generalized estimating equation which provides the basis for computing valid large-sample confidence intervals and test statistics.

There is also a further modification when the data are cluster sampled. The likelihood for cluster sampled data does not have the simple form given above. Instead it involves a product of multiple integrals of conditional likelihoods. Such likelihoods are computationally challenging and the results may be sensitive to the specification of the correlation structure. An alternative approach is to assume the ordinal regression model holds in a population-average sense. Consistent estimates can be obtained quite generally, without making extensive distributional assumptions about the correlation structure. CatReg takes the expression derived above as a "working deviance" criterion. Minimizing it leads to consistent estimates under the population average model. The main impact on the analysis

compared to a standard likelihood analysis is the use of generalized estimating equation methods for making statistical inferences. Rather than reporting the inverse information matrix as the estimated parameter covariance, the sandwich formula is used.

When CatReg uses cluster sampled data, the program uses the weighted independence criterion as an estimating criterion, but computes confidence intervals and hypothesis tests without assuming the criterion is the likelihood. Huber (1967) derived the large-sample theory of maximum likelihood: estimators when the working likelihood is different from the actual likelihood of the data. In the literature on robust statistics, this type of estimator is called an "M-estimator" because it generalizes maximum likelihood. Liang and Zeger (1986) extended the method to the analysis of correlated data, based on a working correlation structure, without assuming the working correlation structure was correct.

APPENDIX E: ESTIMATES & ASSUMPTIONS TO DEFINE COPPER INTAKE & BODYWEIGHT

Table E1: Estimates and Assumptions for Copper Intake and Bodyweight – Copper Excess

ID#	*Weight at T1 (kg)*	*Age at T1 (days)*	*Exposure T (days)*	*Weight at T2*	*Age at T2*	*Age at Midpoint (days)*	*Weight at Midpoint (kg)*	*Consumption Feed (g)*	*Consumption water (ml)*
2	82.2	NA	42	NA	NS	NA	82.2	NA	NA
2	61.3	NA	42	NA	NS	NA	61.3	NA	NA
No need to estimate amount of copper in feed consumed – exposure was given via a capsule with copper content reported in milligrams per day. Weight and age is assumed to be constant from the beginning to the end of the study.									
6	**70**	NA	84	NA	NS	NA	**70**	NA	NA
Weight not given but assumed to be 70 kg. No need to estimate amount of copper in feed consumed – exposure was given via a capsule with copper content reported in milligrams.									
10	0.06	**27**	30	NS	**57**	**42**	**0.165**	**18**	NA
Weight reported at onset. Age and weight at mid-point estimated from Poiley (1972) based on specie, strain and weight. Consumption of feed derived from the NAS (1972) estimates by species, sex and weight.									
14	**70**	NS	28	NA	NA	NS	**70**	NA	NA
Weight at onset assumed to be 70kg for adult male. Weight and age is assumed to be constant from the beginning to the end of the study. Amount of copper consumed provided in the article.									
20	NS	**21**	7	NS	**28**	**24.5**	**0.069**	**9**	NA
20	NS	**21**	14	NS	**35**	**28**	**0.080**	**12**	NA
20	NS	**21**	21	NS	**42**	**31.5**	**0.100**	**15**	NA
20	NS	**21**	28	NS	**49**	**35**	**0.120**	**16**	NA
20	NS	**21**	35	NS	**56**	**38.5**	**0.139**	**17**	NA
20	NS	**21**	42	NS	**63**	**42**	**0.159**	**18**	NA
20	NS	**21**	105	NS	**126**	**73.5**	**0.326**	**20**	NA
Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at midpoint estimated from Poiley (1972) based on specie, strain and age. Consumption of feed based on NAS (1972).									
22	NS	**21**	7	NS	**28**	**24.5**	**0.069**	**9**	**NA**
22	NS	**21**	14	NS	**35**	**28**	**0.080**	**12**	**NA**
22	NS	**21**	21	NS	**42**	**31.5**	**0.997**	**15**	**NA**
22	NS	**21**	42	NS	**63**	**42**	**0.120**	**18**	**NA**

22	NS	**21**	63	NS	**84**	**52.5**	**0.213**	**19**	**NA**
22	NS	**21**	105	NS	**126**	**73.5**	**0.326**	**20**	**NA**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at midpoint estimated from Poiley (1972) based on specie, strain and age. Consumption of feed derived from the NAS (1972).

25	NS	**72**	126	NS	**198**	**135**	**0.312**	**20**	**43.68**
25	NS	**72**	126	NS	**198**	**135**	**0.211**	**16**	**29.54**

Animals reported as being 'adults'. Estimate of 72 days for the age of adult rats derived from the CCAC (1984). Weight at midpoint estimated from Poiley (1972) based on specie, strain and age. Consumption of feed based on NAS (1972) and consumption of water derived from the CCAC (1984).

26	0.083	**36**	15	NA	**51**	**43.5**	**0.105**	NA	NA
26	0.025	**42**	15	NA	**57**	**49.5**	**0.024**	NA	NA
26	0.088	**42**	15	NA	**57**	**49.5**	**0.111**	NA	NA
26	0.02	**35**	15	NA	**50**	**42.5**	**0.023**	NA	NA
26	0.107	**42**	15	NA	**57**	**49.5**	**0.154**	NA	NA
26	0.098	**46**	15	NA	**61**	**53.5**	**0.120**	NA	NA
26	0.022	**36**	15	NA	**51**	**43.5**	**0.023**	NA	NA
26	0.018	**32**	15	NA	**47**	**39.5**	**0.022**	NA	NA
26	0.119	**32**	91	NA	**123**	**77.5**	**0.335**	NA	NA
26	0.106	**31**	91	NA	**122**	**76.5**	**0.240**	NA	NA
26	0.021	**54**	91	NA	**145**	**99.5**	**0.030**	NA	NA
26	0.017	**49**	91	NA	**140**	**94.5**	**0.026**	NA	NA

Age estimated from Poiley (1972) based on specie, strain and body weight. Weight at midpoint estimated from Poiley (1972) based on specie, strain and age. Estimates of feed and water intake not needed as study provides detailed information on amount of copper consumed.

35	**70**	NA	35	NA	NS	NA	**70**	NA	NA

Weight not given but assumed to be 70Kg. No need to estimate the amount of copper in water consumed as exposure estimates are provided in the article.

37	79	30.9	42	NS	NS	NS	79	NA	NA

No need to estimate the amount of copper in water consumed as the exposure estimates are provided in article.

42	0.1	**47**	210	NS	**257**	**152**	**0.222**	**15**	**31.08**
42	0.1	**47**	350	NS	**397**	**222**	**0.292**	**16**	**40.88**

Age estimated from Poiley (1972) based on specie, strain and body weight. Weight at midpoint estimated from Poiley (1972) based on specie, strain and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

43	7.4	28	28	16.7	56	42	**12.05**	70.91	NA

Weight at midpoint estimated from the reported weight at onset and termination. Average consumption reported in the study.

44	**0.0374**	**21**	84	NS	**105**	**63**	**0.179**	**19**	**25.06**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the Canadian Council on Animal Care (CCAC) (1984).

48	NS	**21**	7	NS	28	24.5	**0.069**	**9**	NA
48	NS	**21**	28	NS	49	35	**0.120**	**16**	NA
48	NS	**21**	56	NS	68	44.5	**0.191**	**19**	NA
48	NS	**21**	84	NS	105	63	**0.288**	**20**	NA
48	NS	**21**	112	NS	133	66.5	**0.304**	**20**	NA

Age at onset and mid-point estimated from Poiley (1972) based on specie, strain, sex and body weight. Consumption of feed derived from the NAS (1972).

50	0.0107	**21**	28	NS	49	35	**0.022**	**3.3**	**3.3**

Body weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and body weight. Feed consumption estimates derived from the Louisiana Veterinary Medical Association (LVMA) (2007).

54	70	32	1	NA	35	35	**70**	NA	200

Body weight assumed to be 70kg.

55	0.16	28	112	NS	140	56	**0.233**	**20**	**NA**

Body weight at onset and mid-point estimated from Poiley (1972) based on specie, strain, sex and body weight. Consumption of feed derived from the NAS (1972).

56	0.125	**34**	252	0.372	**NA**	**NA**	**0.249**	**21**	**34.86**

Age at onset and mid-point estimated from Poiley (1972) based on specie, strain, sex and body weight. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

63	NS	**21**	NS	NS	NS	**70**	**70**	NA	NA

Weight assumed to be 70kg

75	**0.139**	35	105	0.322	140	**87.5**	**0.231**	**29.5**	**86**

Body weight at onset and mid-point derived from Poiley (1972) based on specie, strain, sex and body weight. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

80	NS	70	42	NA	112	91	**0.250**	20	NA

Body weight estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972).

82	NS	NS	1095	NA	NS/NA	NS/NA	**70**	NA	NA
Body weight assumed to be 70 kg									
98	**0.256**	56	56	NS	112	84	**0.342**	**20**	**47.88**
Body weight estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).									
99	**0.1**	90	20	NS	110	100	**0.3**	**18**	**42**
Body weight estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).									
103	**0.0154**	30	906	NS	936	483	**0.035**	**5.25**	**5.25**
103	**0.0154**	30	776	NS	806	418	**0.034**	**5.1**	**5.1**
Body weight estimated from Poiley (1972) based on specie, strain, sex and age. Feed and water consumption estimates derived from the LVMA (2007).									
104	64	NA	77	NS	34	NA	64	NA	NA
Weight and age assumed to be constant from the beginning to the end of the study. Amount of copper consumed provided in the article.									
109	NS	NA	1	NA	NA	NA	**70**	NA	200
Weight assumed to be 70kg.									
110	NS	NA	1	NA	NA	NA	**70**	NA	200
Weight assumed to be 70kg.									
111	NS	NA	60	NA	NA	NA	**70**	NA	1500
Weight assumed to be 70kg.									
112	NA	37	60	NA	NA	NA	**70**	NA	1500
Weight assumed to be 70kg.									
114	4.99	17	70	NA	62	48	11.6	538.1	NA
117	0.09	**25**	120	NA	**145**	**85**	**0.300**	**20**	NA
Age at onset was estimated based on body weight from Poiley (1972), weight at midpoint estimated from Poiley (1972) and consumption of feed derived from the NAS (1972).									
126	21.25	NS	30	NS	NS	NS	29.74	610	NA
136	NS	70	133	NA	203	98	**26**	**3.9**	NA
Weight at midpoint estimated from Poiley (1972) and feed consumption estimated from the LVMA (2007).									
138	NS	32	42	NS	NA	NA	71	NA	NA

140	NS	NS	41	NS	NA	NA	**0.256**	**20**	**2.8**
Adult weight assumed to be 256g, feed consumption estimated from the NAS (1972) and water consumption estimated from the CCAC (1984).									
146	NS	38	18	NA	NA	NA	75	NA	NA
152	NA	56	84	NS	140	98	**3**	**110**	NA
Weight and feed consumption derived from the National Laboratory Animal Centre (NLAC) (2001)									
158	NS	60	84	NS	144	102	**25**	**3.75**	NA
Body weight estimated from Poiley (1972) and feed consumption estimated from LVMA (2007).									
172	6	18	10	NS	28	23	**6**	270	NA
Weight at midpoint assumed to be 6kg based on weight at onset and termination.									
178	0.18-0.25	50	20	NS	75	62.5	**0.285**	**20**	NA
Weight at midpoint estimated from Poiley (1972) and feed consumption derived from the NAS (1972).									
180	NS	120	60	NS	180	150	**0.44**	**20**	**61.6**
Weight at midpoint estimated from Poiley (1972). Feed consumption estimated from the NAS (1972). Water consumption estimated from the CCAC (1984).									
187	NS	28	7	NS	35	32	**0.075**	NA	**2.55**
Weight at midpoint estimated from Poiley (1972) and water consumption estimated from the CCAC (1984).									

Note. ID#, identification number corresponds with ref(ID#) in table A1; Exp, experiment number; Weight at T1, body weight at the beginning of the study; Age at T1, age at the Beginning of the study; Exposure T, exposure duration; Weight at T2, weight at the end of the study; Age at T2, age at the end of the study.

Table E2: Estimates and Assumptions for Copper Intake and Bodyweight – Copper Deficiency

ID #	*Weight at T1 (kg)*	*Age at T1 (days)*	*Exposure T (days)*	*Weight at T2*	*Age at T2*	*Age at Midpoint*	*Weight at Midpoint (kg)*	*Consumption Feed (g)*	*Consumption water (ml)*
1	NS	0	18	NS	18	9	**0.005**	**2.85**	**2.85**
Body weight estimated from Poiley (1972) based on specie, strain, sex and age. Feed and water consumption estimates derived from the LVMA (2007)									
4	**0.0374**	**21**	42	NS	**63**	**42**	**0.098**	**18**	**13.65**
Animals reported as being in the weaning stage. Estimate of 21 days for the age of a weaning rat derived from the CCAC (1984). Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).									
8	0.034	**168**	100	NS	**268**	**218**	**0.036**	**5.4**	**5.4**
Age at onset estimated from Poiley (1972) based on specie, strain, sex and body weight. Weight at mid-point estimated from Poiley (1972) based on specie, strain, sex and age.									
16	**0.0594**	**21**	28	NS	**49**	**35**	**0.13**	**17**	**18.2**
Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the NAS (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed based on NAS (1972) and consumption of water based on the CCAC (1984).									
17	**0.0594**	**21**	7	NS	**28**	**24.5**	**0.079**	**12**	**11.088**
17	**0.0594**	**21**	21	NS	**42**	**31.5**	**0.115**	**16**	**16.1**
17	**0.0594**	**21**	35	NS	**56**	**38.5**	**0.147**	**17**	**20.58**
Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).									
18	74	26	24	NS	NA	26	74	NA	NA
Weight and age is assumed to be constant from the beginning to the end of the experiment. Amount of copper consumed provided in the article.									
19	0.099	17.5	38.5	NS	56	36.75	**0.14**	**17**	**19.6**
Weight at mid-point estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).									
24	**0.0594**	**21**	35	NS	**56**	**38.5**	**0.147**	**17**	**20.58**
Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).									
27	**0.0436**	49	60	NS	109	79	**0.025**	**3.69**	**3.69**
Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Feed and water consumption estimates derived from the LVMA (2007).									
31	**70**	22-35	90	NS	22-35	22-35	70	NA	NA

Weight at onset assumed to be 70kg for adult male. Weight and age is assumed to be constant from the beginning to the end of the study. Amount of copper consumed provided in the article.

32	**0.018**	**21**	140	NS	**161**	**91**	**0.373**	**20**	**52.178**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

33	0.046	**21**	63	NS	**84**	**52.5**	**0.216**	**21**	**30.24**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the Canadian Council on Animal Care (CCAC) (1984).

34	**0.057**	**21**	49	NS	**70**	**45.5**	**0.167**	**18**	**23.352**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

37	79	20-59	42	NS	NA	NA	79	NA	NA

Weight and age is assumed to be constant from the beginning to the end of the study. Amount of copper consumed provided in the article.

38	**0.0533**	21	35	NS	56	38.5	**0.161**	**17**	**22.54**
38	NA	0	56	NS	56	28	**0.096**	**13.5**	**13.454**

Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

39	6	21	77	46	98	**59.5**	**20**	**554**	NA

Weight at onset and termination given. Weight at midpoint estimated from Queensland Government 2005. Consumption of feed derived from Cromwell (1989).

40	**0.057**	**21**	28	NS	49	**35**	**0.1326**	**16**	**18.564**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

41	0.11	35	84	NS	119	77	**0.223**	**21**	**31.22**

Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

44	**0.0374**	**21**	84	NS	**105**	**63**	**0.179**	**19**	**25.06**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

45	**0.0374**	**21**	35	NS	**56**	**38.5**	**0.161**	**17**	**22.54**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

47	0.0425	**21**	28	NS	**49**	**35**	**0.127**	**15**	**17.71**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

50	0.01065	21	28	NS	49	35	**0.027**	**4.065**	**4.065**

Weight at midpoint estimated from Poiley (1872) based on specie, strain, sex and age. Feed and water consumption estimates derived from the LVMA (2007).

51	**0.27**	**72**	28	NS	**100**	**86**	**0.304**	**18**	**42.56**

Animals reported as being adults. Estimate of 72 days for adult rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age.

52	**0.238**	180	70	NS	250	215	**0.271**	10.1	**NA**
52	**0.325**	720	70	NS	790	755	**0.325**	13.7	**NA**

Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Amount of copper consumed provided in the article.

53	**0.023**	10	60	NS	70	40	**0.108**	**15**	**15.12**

Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

55	**0.0989**	28	112	NS	140	84	**0.358**	**20**	**50.12**

Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

58	**0.0941**	21	98	NS	119	70	**0.02276**	**3.41**	**3.41**

Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Feed and water consumption derived from the LVMA (2007).

62	**0.0575**	**21**	161	NS	**182**	**101.5**	**0.315**	**18**	**44.1**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

66	**0.0594**	21	35	NS	56	38.5	0.161	**18**	**22.54**

Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

67	0.011	21	35	NS	56	**38.5**	**0.02**	**3**	**3**

Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Feed and water consumption estimates derived from the LVMA (2007).

68	**0.011**	21	21	NS	42	31.5	**0.01675**	**2.5**	**2.5**

Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Feed and water consumption estimates derived from the LVMA (2007).

70	**0.01803**	**21**	35	NS	**56**	**38.5**	**0.161**	**17**	**22.54**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

71	70	NS	150	NS	NS	NS	70	NA	NA

Age assumed to be 70kg. Weight and age is assumed to be constant from the beginning to the end of the study. Amount of copper consumed provided in the article.

72	0.05	**21**	28	NS	**49**	**35**	**0.133**	**16**	**18.62**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

73	**0.057**	**21**	28	NS	**49**	**35**	**0.133**	**16**	**18.62**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

74	**0.057**	**21**	28	NS	49	35	**0.133**	**16**	**18.62**

Weanling rats assumed to be 21 days of age (NAS 1969). Weight at onset and midpoint estimated from Poiley 1972. Feed consumption estimated from the NAS (1972) and water consumption estimated from CCAC (1984).

76	**0.0178**	42	49	NS	91	66.5	**0.0221**	**3.32**	**3.32**

Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Feed and water consumption estimates derived from the LVMA (2007).

77	**0.057**	21	77	NS	98	59.5	**0.249**	**19**	**34.86**

Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

78	**0.04**	**21**	84	NS	105	63	**0.258**	**20**	**36.12**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

81	0.042	**21**	14	NS	**35**	**28**	**0.0985**	**14**	**13.79**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

83	**0.057**	**21**	21	NS	**42**	**31.5**	**0.116**	**15**	**16.24**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

84	**0.057**	**21**	30	NS	**51**	**36**	**0.137**	**18**	**19.18**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

85	**0.057**	21	35	NS	56	**56**	**0.233**	**20**	**32.62**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

86	**0.017**	7	28	NS	35	21	**0.058**	9	NA

Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972)

89	0.06	**21**	42	NS	**63**	**42**	**0.181**	**19**	**25.34**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

90	**0.057**	**21**	35	NS	**106**	**63.5**	**0.268**	**20**	**37.52**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

91	NS	**21**	35	NS	56	45.5	**0.1807**	**19**	NA

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972).

92	**0.129**	**40**	35	NS	**75**	**57.5**	**0.199**	**18**	**27.86**

Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

95	**0.057**	21	24	NS	45	33	**0.1**	**15**	**14**

Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

96	**0.057**	**21**	154	NS	**175**	**98**	**0.377**	**20**	**52.78**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

100	**0.0533**	**21**	42	NS	**63**	**42**	**0.181**	**19**	**25.34**

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

102	70	**21-57**	77	NA	NA	NA	70	NA	NA

Age assumed to be 70kg. Weight and age is assumed to be constant from the beginning to the end of the study. Amount of copper consumed provided in the article.

106	0.043	21	168	NS	189	105	**0.381**	**20**	**53.34**

Weight at onset and midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) and consumption of water derived from the CCAC (1984).

107	0.054	28	42	NS	70	49	**0.175**	**19**	NA

Weight at midpoint estimated from Poiley (1972) and consumption estimated from NAS 1972.

108	NS	49	49	NS	98	74	**0.250**	**20**	NA

Body weight estimated from Poiley (1972) and feed consumption estimated from the NAS 1972.

115	NS	56	84	NS	140	126	**0.027**	**4.05**	NA

Weight at midpoint estimated from Poiley (1972) and feed consumption estimated from the LVMA (2007).

118	NS	**21**	28	NS	**51**	**36**	**0.12**	**17**	NA

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972).

119	NS	**21**	30	NS	**51**	**36**	**0.120**	17.8	NA

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age.

120	NS	21	30	NS	51	36	**0.125**	**17**	NA

Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) estimates.

121	NS	21	28	NS	52	37	**0.085**	**13**	NA

Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) estimates.

122	NS	NA	42	NS	NA	NA	0.087	NA	NA

Information on copper intake and body weight provided in the article.

123	NS	21	35	NS	56	38.5	**0.130**	**17**	NA

Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) estimates.

125	0.25	56	180	NS	236	146	**0.440**	**20**	NA

Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972).

129	NS	NA	56	NA	NA	NA	78.4	NA	NA

Information on copper intake and body weight provided in the article.

133	NS	21	42	NS	63	42	**0.167**	**18**	NA
133	NS	21	42	NS	63	42	**0.155**	**15**	NA

Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972).

137	NS	70	470	NS	540	305	**0.5**	**22**	NA

Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed based on NAS (1972) estimates.

141	NS	72	35	NS	113	93	**0.250**	**20**	**37.5**
141	NS	42	35	NS	87	65	**0.200**	**3**	**3**

Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed for rats derived from the NAS (1972) estimates and water consumption based from CCAC 1984. Consumption of feed and water derived from the LVMA (2007).

142	NS	**21**	35	NS	56	39	**0.125**	**16**	NA

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the NAS (1969). Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) estimates.

143	NS	**21**	35	NS	56	39	**0.125**	**16**	NA

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972).

144	NS	**21**	28	NS	49	35	**0.125**	**17**	NA

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972).

148	NS	28	60	NS	88	58	**0.232**	**21**	NA

Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed based on NAS (1972) estimates.

149	NS	21	35	NS	56	39	**0.155**	**17**	NA

Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972) estimates.

167	NS	**21**	35	NS	56	38.5	**0.1**	**15**	NA

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972).

177	NS	**21**	28	NS	**49**	**35**	**0.125**	**17**	NA

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972).

179	NS	**21**	28	NS	**49**	**35**	**0.125**	**16**	NA

Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats derived from the CCAC (1984). Weight at midpoint estimated from Poiley (1972)

based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972).									
183	NS	**21**	35	NS	**56**	**38.5**	**0.125**	**16**	NA
Animals reported as being in the weaning stage. Estimate of 21 days for the age of weaning rats taken from NAS (1969). Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed based on NAS (1972) estimates.									
185	NS	24	35	NS	59	42	**0.152**	**17**	NA
Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972).									
202	NS	21	19	NS	40	31	**0.125**	**17**	NA
202	NS	21	19	NS	40	31	**0.115**	**16**	NA
Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed derived from the NAS (1972).									
211	NS	165	49	NS	214	190	**0.5**	**21**	NA
Weight at midpoint estimated from Poiley (1972) based on specie, strain, sex and age. Consumption of feed based on NAS (1972) estimates.									

Note. ID#, identification number corresponds with ref(ID#) in table A2; Exp, experiment number; Weight at T1, body weight at the beginning of the study; Age at T1, age at the beginning of the study; Exposure T, exposure duration; Weight at T2, weight at the end of the study; Age at T2, age at the end of the study.

TABLE E3: Final Estimates for 5 Dose Metrics – Copper Excess

ID#	*Exp*	*Grp*	*Base*	*Metric*	*Feed Consump. (kg)*	*Water Consump. (mL)*	*Mg/d*	*bw*	*$bw^{1/4}$*	*$bw^{2/3}$*	*$bw^{3/4}$*	*mg/bw*	*$mg/bw^{1/4}$*	*$mg/bw^{2/3}$*	*$mg/bw^{3/4}$*
2	1	1	1.4	mg/d	NA	NA	1.4	82.2	0.4650	18.9052	27.2994	0.0170	0.4650	0.0741	0.0513
2	1	2	4.4	mg/d	NA	NA	4.4	82.2	1.4613	18.9052	27.2994	0.0535	1.4613	0.2327	0.1612
2	1	3	7.4	mg/d	NA	NA	7.4	82.2	2.4576	18.9052	27.2994	0.0900	2.4576	0.3914	0.2711
2	2	1	1.1	mg/d	NA	NA	1.1	61.3	0.3931	15.5468	21.9076	0.0179	0.3931	0.0708	0.0502
2	2	2	4.1	mg/d	NA	NA	4.1	61.3	1.4653	15.5468	21.9076	0.0669	1.4653	0.2637	0.1871
2	2	3	7.1	mg/d	NA	NA	7.1	61.3	2.5374	15.5468	21.9076	0.1158	2.5374	0.4567	0.3241
Habitual dietary Cu intake for males and females provided in the article (males =1.4 mg/day, females = 1.1 mg/day)															
6	1	1	1.25	mg/d	NA	NA	1.25	70	2.8925	16.9850	24.2005	0.0179	0.4322	0.0736	0.0517
6	1	2	6.25	mg/d	NA	NA	6.25	70	2.8925	16.9850	24.2005	0.1607	3.8894	0.6623	0.4649
Habitual dietary Cu estimated to be 1.25 mg/day (Baker 1999a)															
10	1	1	5.6	mg/kg f	0.018	NA	0.1008	0.165	0.6373	0.3008	0.2589	0.6109	0.1582	0.3351	0.3894
10	1	2	250	mg/kg f	0.018	NA	4.5	0.165	0.6373	0.3008	0.2589	27.2727	7.0606	14.9586	17.3820
Average levels of copper in feed assumed to be 5.6 mg/kg feed/day (NAS 1972)															
14	1	1	0	mg/d	NA	NA	1.4	70	2.8925	16.9850	24.2005	0.0200	0.4840	0.0824	0.0579
14	1	2	2	mg/d	NA	NA	3.4	70	2.8925	16.9850	24.2005	0.0486	1.1755	0.2002	0.1405
Habitual dietary Cu estimated to be 1.4mg/day (Baker 1999a)															
20	1	1	10	mg/kg	0.009	NA	0.09	0.0692	0.5129	0.1686	0.1350	1.3000	0.1755	0.5338	0.6668
20	1	2	3000	mg/kg	0.009	NA	27	0.0692	0.5129	0.1686	0.1350	390.0043	52.6369	160.1404	200.0521
20	1	3	4000	mg/kg	0.009	NA	36	0.0692	0.5129	0.1686	0.1350	520.0058	70.1825	213.5205	266.7361
20	1	4	5000	mg/kg	0.009	NA	45	0.0692	0.5129	0.1686	0.1350	650.0072	87.7281	266.9006	333.4201
20	1	5	6000	mg/kg	0.009	NA	54	0.0692	0.5129	0.1686	0.1350	780.0087	105.2738	320.2808	400.1041
20	2	1	10	mg/kg	0.012	NA	0.12	0.0799	0.5316	0.1854	0.1502	1.5026	0.2257	0.6471	0.7988
20	2	2	3000	mg/kg	0.012	NA	36	0.0799	0.5316	0.1854	0.1502	450.7889	67.7205	194.1257	239.6379
20	2	3	4000	mg/kg	0.012	NA	48	0.0799	0.5316	0.1854	0.1502	601.0518	90.2940	258.8342	319.5172
20	2	4	5000	mg/kg	0.012	NA	60	0.0799	0.5316	0.1854	0.1502	751.3148	112.8675	323.5428	399.3965
20	2	5	6000	mg/kg	0.012	NA	72	0.0799	0.5316	0.1854	0.1502	901.5778	135.4410	388.2513	479.2758

20	3	1	10	mg/kg	0.015	NA	0.15	0.0997	0.5619	0.2150	0.1774	1.5048	0.2670	0.6977	0.8455
20	3	2	3000	mg/kg	0.015	NA	45	0.0997	0.5619	0.2150	0.1774	451.4446	80.0867	209.3183	253.6626
20	3	3	4000	mg/kg	0.015	NA	60	0.0997	0.5619	0.2150	0.1774	601.9262	106.7823	279.0910	338.2168
20	3	4	5000	mg/kg	0.015	NA	75	0.0997	0.5619	0.2150	0.1774	752.4077	133.4779	348.8638	422.7711
20	3	5	6000	mg/kg	0.015	NA	90	0.0997	0.5619	0.2150	0.1774	902.8892	160.1734	418.6366	507.3253
20	4	1	10	mg/kg	0.016	NA	0.16	0.1195	0.5880	0.2426	0.2032	1.3389	0.2721	0.6595	0.7872
20	4	2	3000	mg/kg	0.016	NA	48	0.1195	0.5880	0.2426	0.2032	401.6736	81.6393	197.8469	236.1649
20	4	3	4000	mg/kg	0.016	NA	64	0.1195	0.5880	0.2426	0.2032	535.5649	108.8524	263.7959	314.8865
20	4	4	5000	mg/kg	0.016	NA	80	0.1195	0.5880	0.2426	0.2032	669.4561	136.0655	329.7449	393.6081
20	4	5	6000	mg/kg	0.016	NA	96	0.1195	0.5880	0.2426	0.2032	803.3473	163.2786	395.6938	472.3298
20	5	1	10	mg/kg	0.017	NA	0.17	0.1393	0.6109	0.2687	0.2280	1.2204	0.2783	0.6326	0.7456
20	5	2	3000	mg/kg	0.017	NA	51	0.1393	0.6109	0.2687	0.2280	366.1163	83.4800	189.7883	223.6695
20	5	3	4000	mg/kg	0.017	NA	68	0.1393	0.6109	0.2687	0.2280	488.1551	111.3067	253.0511	298.2260
20	5	4	5000	mg/kg	0.017	NA	85	0.1393	0.6109	0.2687	0.2280	610.1938	139.1333	316.3138	372.7826
20	5	5	6000	mg/kg	0.017	NA	102	0.1393	0.6109	0.2687	0.2280	732.2326	166.9600	379.5766	447.3391
20	6	1	10	mg/kg	0.018	NA	0.18	0.1592	0.6317	0.2937	0.2520	1.1307	0.2850	0.6128	0.7142
20	6	2	3000	mg/kg	0.018	NA	54	0.1592	0.6317	0.2937	0.2520	339.1960	85.4886	183.8365	214.2577
20	6	3	4000	mg/kg	0.018	NA	72	0.1592	0.6317	0.2937	0.2520	452.2613	113.9847	245.1153	285.6769
20	6	4	5000	mg/kg	0.018	NA	90	0.1592	0.6317	0.2937	0.2520	565.3266	142.4809	306.3941	357.0962
20	6	5	6000	mg/kg	0.018	NA	108	0.1592	0.6317	0.2937	0.2520	678.3920	170.9771	367.6730	428.5154
20	7	1	10	mg/kg	0.02	NA	0.2	0.3258	0.7555	0.4735	0.4312	0.6139	0.2647	0.4224	0.4638
20	7	2	3000	mg/kg	0.02	NA	60	0.3258	0.7555	0.4735	0.4312	184.1621	79.4170	126.7216	139.1355
20	7	3	4000	mg/kg	0.02	NA	80	0.3258	0.7555	0.4735	0.4312	245.5494	105.8893	168.9621	185.5140
20	7	4	5000	mg/kg	0.02	NA	100	0.3258	0.7555	0.4735	0.4312	306.9368	132.3617	211.2026	231.8925
20	7	5	6000	mg/kg	0.02	NA	120	0.3258	0.7555	0.4735	0.4312	368.3241	158.8340	253.4431	278.2710
22	1	1	5.6	mg/kg	0.009	NA	0.0504	0.06926	0.5130	0.1687	0.1350	0.7277	0.0982	0.2988	0.3733
22	1	2	2000	mg/kg	0.009	NA	18	0.06926	0.5130	0.1687	0.1350	259.8903	35.0875	106.7294	133.3247
22	2	1	5.6	mg/kg	0.012	NA	0.0672	0.07986	0.5316	0.1854	0.1502	0.8415	0.1264	0.3624	0.4473
22	2	2	2000	mg/kg	0.012	NA	24	0.07986	0.5316	0.1854	0.1502	300.5259	45.1470	129.4171	159.7586
22	3	1	5.6	mg/kg	0.015	NA	0.084	0.09968	0.5619	0.2150	0.1774	0.8427	0.1495	0.3907	0.4735
22	3	2	2000	mg/kg	0.015	NA	30	0.09968	0.5619	0.2150	0.1774	300.9631	53.3911	139.5455	169.1084
22	4	1	5.6	mg/kg	0.018	NA	0.1008	0.1195	0.5880	0.2426	0.2032	0.8435	0.1714	0.4155	0.4959
22	4	2	2000	mg/kg	0.018	NA	36	0.1195	0.5880	0.2426	0.2032	301.2552	61.2295	148.3852	177.1237

22	5	1	5.6	mg/kg	0.019	NA	0.1064	0.2128	0.6792	0.3564	0.3133	0.5000	0.1567	0.2985	0.3396
22	5	2	2000	mg/kg	0.019	NA	38	0.2128	0.6792	0.3564	0.3133	178.5714	55.9488	106.6111	121.2844
22	6	1	5.6	mg/kg	0.02	NA	0.112	0.3258	0.7555	0.4735	0.4312	0.3438	0.1482	0.2365	0.2597
22	6	2	2000	mg/kg	0.02	NA	40	0.3258	0.7555	0.4735	0.4312	122.7747	52.9447	84.4810	92.7570
Average levels of copper in feed assumed to be 5.6 mg/kg feed/day (NAS 1972)															
25	1	1	5.6	ppm	0.02	NA	0.112	0.312	0.7474	0.4600	0.4175	0.3590	0.1499	0.2435	0.2683
25	1	2	1505.6	ppm	0.02	NA	30.112	0.312	0.7474	0.4600	0.4175	96.5128	40.2903	65.4591	72.1313
25	2	1	5.6	ppm	0.016	NA	0.0896	0.211	0.6778	0.3544	0.3113	0.4246	0.1322	0.2528	0.2878
25	2	2	1505.6	ppm	0.016	NA	24.0896	0.211	0.6778	0.3544	0.3113	114.1687	35.5434	67.9685	77.3780
Average levels of copper in feed assumed to be 5.6 mg/kg feed/day (NAS 1972)															
26	1	1	0	ppm	NA	25.3	0.0003	0.181	0.6523	0.3200	0.2775	0.0014	0.0004	0.0008	0.0009
26	1	2	300	ppm	NA	25.3	7.6020	0.181	0.6523	0.3200	0.2775	42.0000	11.6549	23.7579	27.3949
26	1	3	1000	ppm	NA	25.3	25.3400	0.181	0.6523	0.3200	0.2775	140.0000	38.8496	79.1931	91.3162
26	1	4	3000	ppm	NA	25.3	76.0200	0.181	0.6523	0.3200	0.2775	420.0000	116.5489	237.5794	273.9485
26	1	5	10000	ppm	NA	25.3	253.4000	0.181	0.6523	0.3200	0.2775	1400.00	388.4964	791.9314	913.1617
26	1	6	30000	ppm	NA	25.3	760.2000	0.181	0.6523	0.3200	0.2775	4200.0000	1165.4891	2375.7942	2739.4850
26	2	1	0	ppm	NA	3.75	0.0001	0.025	0.3976	0.0855	0.0629	0.0015	0.0001	0.0004	0.0006
26	2	2	300	ppm	NA	3.75	1.125	0.025	0.3976	0.0855	0.0629	45.0000	2.8292	13.1581	17.8936
26	2	3	1000	ppm	NA	3.75	3.75	0.025	0.3976	0.0855	0.0629	150.0000	9.4308	43.8603	59.6453
26	2	4	3000	ppm	NA	3.75	11.25	0.025	0.3976	0.0855	0.0629	450.0000	28.2923	131.5808	178.9359
26	2	5	10000	ppm	NA	3.75	37.5	0.025	0.3976	0.0855	0.0629	1500.0000	94.3075	438.6027	596.4530
26	2	6	30000	ppm	NA	3.75	112.5	0.025	0.3976	0.0855	0.0629	4500.0000	282.9225	1315.8080	1789.3491
26	3	1	0	ppm	NA	17.5	0.0031	0.125	0.5946	0.2500	0.2102	0.0248	0.0052	0.0124	0.0147
26	3	2	300	ppm	NA	17.5	5.25	0.125	0.5946	0.2500	0.2102	42.0000	8.8294	21.0000	24.9733
26	3	3	1000	ppm	NA	17.5	17.5	0.125	0.5946	0.2500	0.2102	140.0000	29.4314	70.0000	83.2445
26	3	4	3000	ppm	NA	17.5	52.5	0.125	0.5946	0.2500	0.2102	420.0000	88.2941	210.0000	249.73335
26	3	5	10000	ppm	NA	17.5	175	0.125	0.5946	0.2500	0.2102	1400.0000	294.3137	700.0000	832.4450
26	3	6	30000	ppm	NA	17.5	525	0.125	0.5946	0.2500	0.2102	4200.0000	882.9412	2100.0000	2497.3349
26	4	1	0	ppm	NA	3.6	0.00004	0.024	0.3936	0.0832	0.0610	0.0015	0.0001	0.0004	0.0006
26	4	2	300	ppm	NA	3.6	1.08	0.024	0.3936	0.0832	0.0610	45.0000	2.7439	12.9802	17.7119
26	4	3	1000	ppm	NA	3.6	3.6	0.024	0.3936	0.0832	0.0610	150.0000	9.1464	43.2675	59.0397
26	4	4	3000	ppm	NA	3.6	10.8	0.024	0.3936	0.0832	0.0610	450.0000	27.4392	129.8025	177.1191

26	4	5	10000	ppm	NA	3.6	36	0.024	0.3936	0.0832	0.0610	1500.0000	91.4639	432.6749	590.3969
26	4	6	30000	ppm	NA	3.6	108	0.024	0.3936	0.0832	0.0610	4500.0000	274.2917	1298.0246	1771.1907
26	5	1	0	ppm	NA	NA	0.2431	0.154	0.6264	0.2873	0.2458	1.5786	0.3881	0.8461	0.9889
26	5	2	1000	ppm	NA	NA	13.156	0.154	0.6264	0.2873	0.2458	85.4286	21.0012	45.7906	53.5160
26	5	3	2000	ppm	NA	NA	25.74	0.154	0.6264	0.2873	0.2458	167.1429	41.0893	89.5904	104.7051
26	5	4	4000	ppm	NA	NA	51.909	0.154	0.6264	0.2873	0.2458	337.0714	82.8633	180.6739	211.1554
26	5	5	8000	ppm	NA	NA	111.111	0.154	0.6264	0.2873	0.2458	721.5000	177.3687	386.7318	451.9772
26	5	6	16000	ppm	NA	NA	182.325	0.154	0.6264	0.2873	0.2458	1183.9286	291.0490	634.5985	741.6614
26	6	1	0	ppm	NA	NA	0.150865	0.12	0.5886	0.2433	0.2039	1.2572	0.2563	0.6201	0.7400
26	6	2	1000	ppm	NA	NA	9.706	0.12	0.5886	0.2433	0.2039	80.8833	16.4909	39.8951	47.6052
26	6	3	2000	ppm	NA	NA	18.99	0.12	0.5886	0.2433	0.2039	158.2500	32.2649	78.0556	93.1406
26	6	4	4000	ppm	NA	NA	38.2965	0.12	0.5886	0.2433	0.2039	319.1375	65.0674	157.4122	187.8335
26	6	5	8000	ppm	NA	NA	81.9735	0.12	0.5886	0.2433	0.2039	683.1125	139.2766	336.9401	402.0569
26	6	6	16000	ppm	NA	NA	134.5125	0.12	0.5886	0.2433	0.2039	1120.9375	228.5427	552.8939	659.7459
26	7	1	0	ppm	NA	NA	0.03588	0.023	0.3894	0.0809	0.0591	1.5600	0.0921	0.4436	0.6075
26	7	2	1000	ppm	NA	NA	3.864	0.023	0.3894	0.0809	0.0591	168.0000	9.9221	47.7770	65.4246
26	7	3	2000	ppm	NA	NA	8.326	0.023	0.3894	0.0809	0.0591	362.0000	21.3798	102.9480	140.9745
26	7	4	4000	ppm	NA	NA	17.779	0.023	0.3894	0.0809	0.0591	773.0000	45.6536	219.8309	301.0312
26	7	5	8000	ppm	NA	NA	26.542	0.023	0.3894	0.0809	0.0591	1154.0000	68.1556	328.1822	449.4049
26	7	6	16000	ppm	NA	NA	64.791	0.023	0.3894	0.0809	0.0591	2817.0000	166.3730	801.1173	1097.0308
26	8	1	0	ppm	NA	NA	0.04416	0.022	0.3851	0.0785	0.0571	2.0073	0.1147	0.5624	0.7731
26	8	2	1000	ppm	NA	NA	3.864	0.022	0.3851	0.0785	0.0571	175.6364	10.0330	49.2140	67.6426
26	8	3	2000	ppm	NA	NA	8.326	0.022	0.3851	0.0785	0.0571	378.4545	21.6188	106.0445	145.7536
26	8	4	4000	ppm	NA	NA	17.779	0.022	0.3851	0.0785	0.0571	808.1364	46.1638	226.4430	311.2364
26	8	5	8000	ppm	NA	NA	26.542	0.022	0.3851	0.0785	0.0571	1206.4545	68.9173	338.0533	464.6400
26	8	6	16000	ppm	NA	NA	64.791	0.022	0.3851	0.0785	0.0571	2945.0455	168.2322	825.2133	1134.2210
26	9	1	0	ppm	NA	NA	0.103	0.335	0.7608	0.4824	0.4403	0.3075	0.1354	0.2135	0.2339
26	9	2	500	ppm	NA	NA	10.3	0.335	0.7608	0.4824	0.4403	30.7463	13.5387	21.3537	23.3913
26	9	3	1000	ppm	NA	NA	18.952	0.335	0.7608	0.4824	0.4403	56.5731	24.9112	39.2909	43.0399
26	9	4	2000	ppm	NA	NA	37.08	0.335	0.7608	0.4824	0.4403	110.6866	48.7392	76.8735	84.2085
26	9	5	4000	ppm	NA	NA	74.778	0.335	0.7608	0.4824	0.4403	223.2179	98.2907	155.0282	169.8205
26	9	6	8000	ppm	NA	NA	160.062	0.335	0.7608	0.4824	0.4403	477.7970	210.3909	331.8372	363.5002
26	10	1	0	ppm	NA	NA	0.11169	0.24	0.6999	0.3862	0.3429	0.4654	0.1596	0.2892	0.3257

26	10	2	500	ppm	NA	NA	7.65	0.24	0.6999	0.3862	0.3429	31.8750	10.9297	19.8086	22.3102
26	10	3	1000	ppm	NA	NA	14.076	0.24	0.6999	0.3862	0.3429	58.6500	20.1107	36.4478	41.0507
26	10	4	2000	ppm	NA	NA	27.54	0.24	0.6999	0.3862	0.3429	114.7500	39.3470	71.3110	80.3166
26	10	5	4000	ppm	NA	NA	55.539	0.24	0.6999	0.3862	0.3429	231.4125	79.3497	143.8105	161.9719
26	10	6	8000	ppm	NA	NA	118.881	0.24	0.6999	0.3862	0.3429	495.3375	169.8477	307.8258	346.7001
26	11	1	0	ppm	NA	NA	0.0318	0.03	0.4162	0.0965	0.0721	1.0600	0.0764	0.3294	0.4411
26	11	2	1000	ppm	NA	NA	5.04	0.03	0.4162	0.0965	0.0721	168.0000	12.1102	52.2015	69.9181
26	11	3	2000	ppm	NA	NA	10.86	0.03	0.4162	0.0965	0.0721	362.0000	26.0945	112.4818	150.6569
26	11	4	4000	ppm	NA	NA	23.19	0.03	0.4162	0.0965	0.0721	773.0000	55.7212	240.1891	321.7065
26	11	5	8000	ppm	NA	NA	34.62	0.03	0.4162	0.0965	0.0721	1154.0000	83.1853	358.5746	480.2707
26	11	6	16000	ppm	NA	NA	84.51	0.03	0.4162	0.0965	0.0721	2817.0000	203.0616	875.3074	1172.3767
26	12	1	0	ppm	NA	NA	0.03321	0.026	0.4016	0.0878	0.0647	1.2773	0.0827	0.3784	0.5129
26	12	2	1000	ppm	NA	NA	4.536	0.026	0.4016	0.0878	0.0647	174.4615	11.2961	51.6842	70.0556
26	12	3	2000	ppm	NA	NA	9.774	0.026	0.4016	0.0878	0.0647	375.9231	24.3405	111.3671	150.9532
26	12	4	4000	ppm	NA	NA	20.871	0.026	0.4016	0.0878	0.0647	802.7308	51.9756	237.8087	322.3393
26	12	5	8000	ppm	NA	NA	31.158	0.026	0.4016	0.0878	0.0647	1198.3846	77.5937	355.0210	481.2154
26	12	6	16000	ppm	NA	NA	76.059	0.026	0.4016	0.0878	0.0647	2925.3462	189.4119	866.6326	1174.6828
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
35	1	1	0	mg/L	NA	200	0.002	70	2.8925	16.9850	24.2005	0.0001	0.0007	0.0001	0.0001
35	1	2	2	mg/L	NA	200	0.4	70	2.8925	16.9850	24.2005	0.0057	0.1383	0.0236	0.0165
35	1	3	4	mg/L	NA	200	0.8	70	2.8925	16.9850	24.2005	0.0114	0.2766	0.0471	0.0331
35	1	4	6	mg/L	NA	200	1.2	70	2.8925	16.9850	24.2005	0.0171	0.4149	0.0707	0.0496
35	1	5	8	mg/L	NA	200	1.6	70	2.8925	16.9850	24.2005	0.0229	0.5532	0.0942	0.061
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
37	1	1	1.6	mg/day	NA	NA	1.6	79	2.9813	18.4113	1.4226	0.0203	0.5367	0.0869	1.4226
37	1	2	6	mg/day	NA	NA	6	79	2.9813	18.4113	3.8337	0.0759	2.0125	0.3259	3.8337
42	1	1	5	ppm	0.015	NA	0.075	0.222	0.6864	0.3666	0.3234	0.3378	0.1093	0.2046	0.2319
42	1	2	200	ppm	0.015	NA	3	0.222	0.6864	0.3666	0.3234	13.5135	4.3705	8.1825	9.2759
42	2	1	5	ppm	0.016	NA	0.08	0.292	0.7351	0.4401	0.3972	0.2740	0.1088	0.1818	0.2014
42	2	2	200	ppm	0.016	NA	3.2	0.292	0.7351	0.4401	0.3972	10.9589	4.3532	7.2705	8.0559

43	1	1	30	ppm	0.554	NA	16.62	12.05	1.8631	5.2560	6.4676	1.3793	8.9204	3.1621	2.5697
43	1	2	155	ppm	0.554	NA	85.87	12.05	1.8631	5.2560	6.4676	7.1261	46.0887	16.3374	13.2770
43	1	3	280	ppm	0.554	NA	155.12	12.05	1.8631	5.2560	6.4676	12.8730	83.2571	29.5128	23.9843
43	2	1	30	ppm	0.554	NA	16.62	12.05	1.8631	5.2560	6.4676	1.3793	8.9204	3.1621	2.5697
43	2	2	155	ppm	0.554	NA	85.87	12.05	1.8631	5.2560	6.4676	7.1261	46.0887	16.3374	13.2770
43	2	3	280	ppm	0.554	NA	155.12	12.05	1.8631	5.2560	6.4676	12.8730	83.2571	29.5128	23.9843
44	1	1	6	mg/kg f	0.021	NA	0.126	0.264	0.7168	0.4115	0.3683	0.4773	0.1758	0.3062	0.3421
44	1	2	250	mg/kg f	0.021	NA	5.25	0.264	0.7168	0.4115	0.3683	19.8864	7.3242	12.7572	14.2546
48	1	1	20	ppm	0.009	NA	0.18	0.06926	0.5130	0.1687	0.1350	2.5989	0.3509	1.0673	1.3332
48	1	2	1500	ppm	0.009	NA	13.5	0.06926	0.5130	0.1687	0.1350	194.9177	26.3156	80.0471	99.9935
48	2	1	20	ppm	0.016	NA	0.32	0.1195	0.5880	0.2426	0.2032	2.6778	0.5443	1.3190	1.5744
48	2	2	1500	ppm	0.016	NA	24	0.1195	0.5880	0.2426	0.2032	200.8368	40.8196	98.9235	118.0824
48	3	1	20	ppm	0.019	NA	0.38	0.191	0.6611	0.3317	0.2889	1.9895	0.5748	1.1458	1.3153
48	3	2	1500	ppm	0.019	NA	28.5	0.191	0.6611	0.3317	0.2889	149.2147	43.1108	85.9322	98.6438
48	4	1	20	ppm	0.02	NA	0.4	0.2882	0.7327	0.4363	0.3933	1.3879	0.5459	0.9168	1.0169
48	4	2	1500	ppm	0.02	NA	30	0.2882	0.7327	0.4363	0.3933	104.0944	40.9447	68.7583	76.2695
48	5	1	20	ppm	0.02	NA	0.4	0.3044	0.7428	0.4525	0.4098	1.3141	0.5385	0.8840	0.9761
48	5	2	1500	ppm	0.02	NA	30	0.3044	0.7428	0.4525	0.4098	98.5545	40.3887	66.2966	73.2045
50	1	1	498	ppm	0.0033	NA	1.6434	0.022	0.3851	0.0785	0.0571	74.7000	4.2671	20.9312	28.7691
50	1	2	200	ppm	0.0033	NA	0.66	0.249	0.7064	0.3958	0.3525	2.6506	0.9343	1.6675	1.8724
54	1	1	10	mg/L	NA	200	2	70	2.8925	16.9850	24.2005	0.0286	0.6914	0.2278	0.0826
55	1	1	25	ppm	0.02	NA	0.5	0.2326	0.6945	0.3782	0.3349	2.1496	0.7200	1.3220	1.4928
55	1	2	100	ppm	0.02	NA	2	0.2326	0.6945	0.3782	0.3349	8.5985	2.8799	5.2880	5.9714
56	1	1	0	mg/L	0.021	52.5	0.0005	0.375	0.7825	0.5200	0.4792	0.0014	0.0007	0.0010	0.0011
56	1	2	1250	mg/L	0.021	52.5	65.63	0.375	0.7825	0.5200	0.4792	175.0133	83.8677	126.2065	136.9553

Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).

63	1	1	250	g/day	NS	NA	250000	70	2.8925	16.9850	24.2005	3571.4286	86430.1962	14718.8761	10330.3843

75	1	1	18	mg/kg f	0.0281	NA	0.5058	0.231	0.6933	0.3765	0.3332	2.1896	0.7296	1.3435	1.5180
75	1	2	100	mg/kg f	0.031	NA	3.1	0.231	0.6933	0.3765	0.3332	13.4199	4.4716	8.2342	9.3036
80	1	1	10	mg/kg	0.02	NA	0.2	0.2497	0.7069	0.3965	0.3532	0.8010	0.2829	0.5044	0.5662
80	1	2	1500	mg/kg	0.02	NA	30	0.2497	0.7069	0.3965	0.3532	120.1442	42.4391	75.6558	84.9293
82	1	1	45	mg/day	NA	NA	45	70	2.8925	16.9850	24.2005	0.6429	15.5574	2.6494	2.8925
98	1	1	5.6	mg/kg f	0.02	NA	0.112	0.342	0.7647	0.4890	0.4472	0.3275	0.1465	0.2290	0.2504
98	1	2	500	mg/kg f	0.02	NA	171	0.342	0.7647	0.4890	0.4472	500.00	223.6092	349.6595	382.3636
Average levels of copper in feed assumed to be 5.6 mg/kg feed/day (NAS 1972)															
99	1	1	5.6	mg/kg	0.018	NA	0.1008	0.3	0.7401	0.4481	0.4054	0.3360	0.1362	0.2249	0.2487
99	1	2	100	mg/kg	0.018	NA	31.68	0.3	0.7401	0.4481	0.4054	105.6000	42.8060	70.6921	78.1527
Average levels of copper in feed assumed to be 5.6 mg/kg feed/day (NAS 1972)															
103	1	1	0	ppm	0.0052	4	0.0001	0.035	0.4325	0.1070	0.0809	0.0011	0.0001	0.0004	0.0005
103	1	2	317	ppm	0.0052	4	1.2680	0.035	0.4325	0.1070	0.0809	36.2286	2.9316	11.8506	15.6700
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
104	1	1	0	mg/l	NA	1.64	0.0164	64	2.8284	16.0000	22.6274	0.0003	0.0058	0.0010	0.0007
104	1	2	1	mg/l	NA	1.64	1.64	64	2.8284	16.0000	22.6274	0.0256	0.5798	0.1025	0.725
104	1	3	3	mg/l	NA	1.64	4.92	64	2.8284	16.0000	22.6274	0.0769	1.7395	0.3075	0.2174
104	1	4	5	mg/l	NA	1.64	8.2	64	2.8284	16.0000	22.6274	0.1281	2.8991	0.5125	0.3624
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
109	1	1	0	mg/l	NA	200	0.002	70	2.8925	16.9850	24.2005	0.0001	0.0007	0.0001	0.0001
109	1	2	10	mg/l	NA	200	2	70	2.8925	16.9850	24.2005	0.0286	0.6914	0.1178	0.0826
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
110	1	1	0	mg/l	NA	200	0.002	70	2.8925	16.9850	24.2005	0.0003	0.0007	0.0001	0.0008
110	1	2	2	mg/l	NA	200	0.4	70	2.8925	16.9850	24.2005	0.0057	0.1383	0.0236	0.0165
110	1	3	4	mg/l	NA	200	0.8	70	2.8925	16.9850	24.2005	0.0114	0.2766	0.0471	0.0331
110	1	4	6	mg/l	NA	200	1.2	70	2.8925	16.9850	24.2005	0.0171	0.4149	0.0707	0.0496

110	1	5	8	mg/l	NA	200	1.6	70	2.8925	16.9850	24.2005	0.0229	0.5532	0.0942	0.0661
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
111	1	1	0.01	mg/l	NA	1500	0.015	70	2.8925	16.9850	24.2005	0.0002	0.0052	0.0009	0.0006
111	1	2	2	mg/l	NA	1500	3	70	2.8925	16.9850	24.2005	0.0429	1.0372	0.1766	0.1240
111	1	3	4	mg/l	NA	1500	6	70	2.8925	16.9850	24.2005	0.0857	2.0743	0.3533	0.2479
111	1	4	6	mg/l	NA	1500	9	70	2.8925	16.9850	24.2005	0.1286	3.1115	0.5299	0.3719
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
112	1	1	0	mg/l	NA	1500	0.015	70	2.8925	16.9850	24.2005	0.0002	0.0052	0.0009	0.0006
112	1	2	2	mg/l	NA	1500	3	70	2.8925	16.9850	24.2005	0.0429	1.0372	0.1766	0.1240
112	1	3	4	mg/l	NA	1500	6	70	2.8925	16.9850	24.2005	0.0857	2.0743	0.3533	0.2479
112	1	4	5	mg/l	NA	1500	7.5	70	2.8925	16.9850	24.2005	0.1071	2.5929	0.4416	0.3099
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
114	1	1	10	ppm	0.5381	NS	5.38	11.6	1.8455	5.1243	6.2856	0.4638	2.9152	1.0499	0.8559
114	1	2	135	ppm	0.5381	NS	72.64	11.6	1.8455	5.1243	6.2856	6.2621	39.3606	14.1755	11.5567
114	1	3	260	ppm	0.5381	NS	139.91	11.6	1.8455	5.1243	6.2856	12.0612	75.8114	27.3030	22.2590
114	2	1	15	ppm	0.5381	NS	8.07	11.6	1.8455	5.1243	6.2856	0.6957	4.3728	1.5748	1.2839
114	2	2	46	ppm	0.5381	NS	24.75	11.6	1.8455	5.1243	6.2856	2.1336	13.4110	4.8299	3.9376
114	2	3	77	ppm	0.5381	NS	41.43	11.6	1.8455	5.1243	6.2856	3.5716	22.4492	8.0849	6.5913
114	2	4	140	ppm	0.5381	NS	75.33	11.6	1.8455	5.1243	6.2856	6.4940	40.8182	14.7004	11.9846
117	1	1	10	ppm	0.02	NA	0.2	0.3	0.7401	0.4481	0.4054	0.6667	0.2702	0.4463	0.4934
117	1	2	1200	ppm	0.02	NA	24	0.3	0.7401	0.4481	0.4054	80	32.4288	53.5546	59.2066
126	1	1	12.4	mg/kg	0.61	NA	7.56	29.74	2.3353	9.5990	12.7352	0.2542	3.2373	0.7876	0.5936
126	1	2	250	mg/kg	0.61	NA	160.06	29.74	2.3353	9.5990	12.7352	5.3820	68.5405	16.6746	12.5683
126	2	1	12.4	mg/kg	0.61	NA	7.56	29.74	2.3353	9.5990	12.7352	0.2542	3.2373	0.7876	0.5936
126	2	2	50	mg/kg	0.61	NA	38.06	29.74	2.3353	9.5990	12.7352	1.2798	16.2980	3.9650	2.9886
126	2	3	100	mg/kg	0.61	NA	68.56	29.74	2.3353	9.5990	12.7352	2.3053	29.3586	7.1424	5.3835
136	1	1	0	mg/L	0.0039	3.9	0.00004	0.026	0.4016	0.0878	0.0647	0.0015	0.0001	0.0004	0.0006
136	1	2	120	mg/L	0.0039	3.9	0.468	0.026	0.4016	0.0878	0.0647	18.0000	1.1655	5.3325	7.2280

136	1	3	300	mg/L	0.0039	3.9	1.17	0.026	0.4016	0.0878	0.0647	45.0000	2.9137	13.3312	18.0699
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
138	1	1	1.23	mg/d	NA	NA	1.23	71	2.9028	17.1464	24.4593	0.0173	0.4237	0.0717	0.0503
138	1	2	4.23	mg/d	NA	NA	4.23	71	2.9028	17.1464	24.4593	0.0596	1.4572	0.2467	0.1729
138	2	1	1.23	mg/d	NA	NA	1.23	71	2.9028	17.1464	24.4593	0.0173	0.4237	0.0717	0.0503
138	2	2	4.23	mg/d	NA	NA	4.23	71	2.9028	17.1464	24.4593	0.0596	1.4572	0.2467	0.1729
138	3	1	1.23	mg/d	NA	NA	1.23	71	2.9028	17.1464	24.4593	0.0173	0.4237	0.0717	0.0503
138	3	2	7.23	mg/d	NA	NA	7.23	71	2.9028	17.1464	24.4593	0.1018	2.4907	0.4217	0.2956
Habitual dietary Cu intake for males and females provided in the article (males =1.4 mg/day, females = 1.1 mg/day)															
140	1	1	0	µg/mL	0.02	35.8	0.0004	0.256	0.7113	0.4032	0.3599	0.0014	0.0005	0.0009	0.0010
140	1	2	250	µg/mL	0.02	35.8	8.95	0.256	0.7113	0.4032	0.3599	34.9609	12.5824	22.1988	24.8681
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
146	1	1	7.8	mg/d	NA	NA	7.8	75	2.9428	17.7845	25.4857	0.104	2.6505	0.4386	0.3061
152	1	1	3.7	mg/d	0.11	NA	3.7	3	1.3161	2.0801	2.2795	1.2333	2.8114	1.7788	1.6232
152	1	2	350	mg/d	0.11	NA	350	3	1.3161	2.0801	2.2795	116.6667	265.9425	168.2624	153.5420
158	1	1	0	ppm	0.00375	NA	0.00001	0.025	0.3976	0.0855	0.0629	0.0015	0.0001	0.0004	0.0006
158	1	2	2	ppm	0.00375	NA	0.0075	0.025	0.3976	0.0855	0.0629	0.3	0.0189	0.0877	0.1193
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
172	1	1	20	ppm	0.27	NA	5.4	6	1.5651	3.3019	3.8337	0.9000	3.4503	1.6354	1.4086
172	1	2	195	ppm	0.27	NA	52.65	6	1.5651	3.3019	3.8337	8.7750	33.6404	15.9452	13.7336
178	1	1	0	mg/d	0.02	39.9	0.0004	0.285	0.7307	0.4331	0.3901	0.0014	0.0005	0.0009	0.0010
178	1	2	12.12	mg/d	0.02	39.9	12	0.285	0.7307	0.4331	0.3901	42.1053	16.4237	27.7088	30.7643
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
180	1	1	0	mg/l	0.02	61.6	0.0006	0.44	0.8144	0.5785	0.5402	0.0014	0.0008	0.0011	0.0011
180	1	2	100	mg/l	0.02	61.6	6.16	0.44	0.8144	0.5785	0.5402	14	7.5634	10.6483	11.4023
180	1	3	400	mg/l	0.02	61.6	24.64	0.44	0.8144	0.5785	0.5402	56.0000	30.2536	42.5931	45.6091
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															

187	1	1	0	µg/mL	NA	14	0.0001	0.075	0.5233	0.1778	25.4857	0.0019	0.0003	0.0008	0.00001
187	1	2	50	µg/mL	NA	14	0.7	0.075	0.5233	0.1778	25.4857	9.3333	1.3376	3.9360	0.0275
187	1	3	100	µg/mL	NA	14	1.4	0.075	0.5233	0.1778	25.4857	18.6667	2.6752	7.8720	0.0549
187	1	4	200	µg/mL	NA	14	2.8	0.075	0.5233	0.1778	25.4857	37.3333	5.3505	15.7441	0.1099

Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).

Note. ID #, reference identification number corresponds with table A1; Exp, experiment number within the publication; Grp, group number within experiment; Base, reported dose of copper; Feed Consump., feed consumption; Water consump., water consumption; mg/bw, milligrams copper per kilogram body weight; $mg/bw^{1/4}$, milligrams copper per kilogram body $weight^{1/4}$; $mg/bw^{2/3}$, milligrams copper per kilogram $bodyweight^{2/3}$; $mg/bw^{3/4}$, milligrams copper per kilogram $bodyweight^{3/4}$, BW, body weight; Cu, copper; mg/kg f, milligrams of copper per kilogram of feed; mg/d, milligrams of copper per day; ppm, parts per million; mg/kg bw/day, milligrams of copper per kilogram body weight per day; mg/L, milligrams of copper per liter of water.

Table E4: Final Estimates for 5 Dose Metrics – Copper Deficiency

Ref	*Exp*	*Grp*	*Base*	*Metric*	*Food Consump. (kg)*	*Water Consump. (mL)*	*Mg/d*	*bw*	*$bw^{1/4}$*	*$bw^{2/3}$*	*$bw^{¾}$*	*mg/bw*	*$mg/bw^{1/4}$*	*$mg/bw^{2/3}$*	*$mg/bw^{3/4}$*
1	1	1	1	ppm	0.00258	NA	0.00258	0.019	0.3713	0.0712	0.0512	0.1358	0.0069	0.0362	0.0504
1	1	2	10	ppm	0.00285	NA	0.0285	0.019	0.3713	0.0712	0.0512	1.5000	0.0768	0.4003	0.5569
4	1	1	8	ppm	0.018	NA	0.144	0.0975	0.5588	0.2118	0.1745	1.4769	0.2577	0.6798	0.8253
4	1	2	2.5	ppm	0.018	NA	0.045	0.0975	0.5588	0.2118	0.1745	0.4615	0.0805	0.2124	0.2579
4	1	3	0.2	ppm	0.018	NA	0.0036	0.0975	0.5588	0.2118	0.1745	0.0369	0.0064	0.0170	0.0206
8	1	1	0	mg/ml	5.4	5.4	0.0302	0.036	0.4356	0.1090	0.0826	0.8389	0.0693	0.2770	0.3654
8	1	2	0.01	mg/ml	5.4	5.4	0.054	0.036	0.4356	0.1090	0.0826	1.5000	0.1240	0.4953	0.6534
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
16	1	1	0	ppm	0.017	NA	0.005	0.13	0.6005	0.2566	0.2165	0.0418	0.0091	0.0212	0.0251
16	1	2	1.5	ppm	0.017	NA	0.0255	0.13	0.6005	0.2566	0.2165	0.1935	0.0419	0.0980	0.1162
16	1	3	3	ppm	0.017	NA	0.051	0.13	0.6005	0.2566	0.2165	0.3622	0.0784	0.1835	0.2175
16	1	4	6	ppm	0.017	NA	0.102	0.13	0.6005	0.2566	0.2165	0.7715	0.1670	0.3908	0.4633
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
17	1	1	6	ppm	0.012	NA	0.072	0.0792	0.5305	0.1844	0.1493	0.9091	0.1357	0.3904	0.4823
17	1	2	3	ppm	0.012	NA	0.036	0.0792	0.5305	0.1844	0.1493	0.4545	0.0679	0.1952	0.2411
17	1	3	1.5	ppm	0.012	NA	0.018	0.0792	0.5305	0.1844	0.1493	0.2273	0.0339	0.0976	0.1206
17	2	1	1.5	ppm	0.016	NA	0.024	0.115	0.5823	0.2365	0.1975	0.2087	0.0412	0.1015	0.1215
17	2	2	3	ppm	0.016	NA	0.048	0.115	0.5823	0.2365	0.1975	0.4174	0.0824	0.2030	0.2431
17	2	3	6	ppm	0.016	NA	0.096	0.115	0.5823	0.2365	0.1975	0.8348	0.1649	0.4060	0.4861
17	3	1	1.5	ppm	0.017	NA	0.0255	0.147	0.6192	0.2785	0.2374	0.1735	0.0412	0.0916	0.1074
17	3	2	3	ppm	0.017	NA	0.051	0.147	0.6192	0.2785	0.2374	0.3469	0.0824	0.1831	0.2148
17	3	3	6	ppm	0.017	NA	0.102	0.147	0.6192	0.2785	0.2374	0.6939	0.1647	0.3662	0.4296
18	1	1	0.66	mg/d	NA	NA	0.66	74	2.9330	17.6260	25.2304	0.0089	0.2250	0.0374	0.0262

19	1	1	0	mg/L	0.017	0.0196	0.00731	0.14	0.6117	0.2696	0.2289	0.0522	0.0120	0.0271	0.0319
19	1	2	20	mg/L	0.017	0.0196	0.39931	0.14	0.6117	0.2696	0.2289	2.8522	0.6528	1.4810	1.7447
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
24	1	1	6.12	ppm	0.017	NA	0.10404	0.147	0.6192	0.2785	0.2374	0.7078	0.1680	0.3735	0.4382
24	1	2	1.17	ppm	0.017	NA	0.01989	0.147	0.6192	0.2785	0.2374	0.1353	0.0321	0.0714	0.0838
24	1	3	0.83	ppm	0.017	NA	0.01411	0.147	0.6192	0.2785	0.2374	0.0960	0.0228	0.0507	0.0594
24	1	4	0.47	ppm	0.017	NA	0.00799	0.147	0.6192	0.2785	0.2374	0.0544	0.0129	0.0287	0.0337
27	1	1	11	ppm	0.00369	NA	0.04059	0.0246	0.3960	0.0846	0.0621	1.6500	0.1025	0.4799	0.6535
27	1	2	6	ppm	0.00369	NA	0.02214	0.0246	0.3960	0.0846	0.0621	0.9000	0.0559	0.2618	0.3564
27	1	3	5	ppm	0.00369	NA	0.01845	0.0246	0.3960	0.0846	0.0621	0.7500	0.0466	0.2181	0.2970
27	1	4	4	ppm	0.00369	NA	0.01476	0.0246	0.3960	0.0846	0.0621	0.6000	0.0373	0.1745	0.2376
27	1	5	3	ppm	0.00369	NA	0.01107	0.0246	0.3960	0.0846	0.0621	0.4500	0.0280	0.1309	0.1782
27	1	6	2	ppm	0.00369	NA	0.00738	0.0246	0.3960	0.0846	0.0621	0.3000	0.0186	0.0873	0.1188
27	1	7	1	ppm	0.00369	NA	0.00369	0.0246	0.3960	0.0846	0.0621	0.1500	0.0093	0.0436	0.0594
31	1	1	0.785	mg/d	NA	NA	0.785	70	2.8925	16.9850	24.2005	0.0112	0.2714	0.0462	0.0324
31	1	2	1.68	mg/d	NA	NA	1.68	70	2.8925	16.9850	24.2005	0.0240	0.5808	0.0989	0.0694
32	1	1	5.79	mg/kg f	0.02	NA	0.1158	0.3727	0.7813	0.5179	0.4770	0.3107	0.1482	0.2236	0.2428
32	1	2	0.46	mg/kg f	0.02	NA	0.0092	0.3727	0.7813	0.5179	0.4770	0.0247	0.0118	0.0178	0.0193
33	1	1	0.57	mcg/g f	0.021	NA	0.01197	0.216	0.6817	0.3600	0.3168	0.0554	0.0176	0.0333	0.0378
33	1	2	5	mcg/g f	0.021	NA	0.105	0.216	0.6817	0.3600	0.3168	0.4861	0.1540	0.2917	0.3314
34	1	1	0.2	mcg/g f	0.018	NA	0.0036	0.1668	0.6391	0.3030	0.2610	0.0216	0.0056	0.0119	0.0138
34	1	2	10	mcg/g f	0.018	NA	0.18	0.1668	0.6391	0.3030	0.2610	1.0791	0.2817	0.5940	0.6896
37	1	1	1.6	mg/d	NA	NA	1.6	79	2.9813	18.4113	26.4984	0.0203	0.5367	0.0869	0.0604
37	1	2	0.7	mg/d	NA	NA	0.7	79	2.9813	18.4113	26.4984	0.0089	0.2348	0.0380	0.0264
38	1	1	0.6	µg/g f	0.017	NA	0.0102	0.161	0.6334	0.2959	0.2542	0.0634	0.0161	0.0345	0.0401

38	1	2	6	µg/g f	0.017	NA	0.102	0.161	0.6334	0.2959	0.2542	0.6335	0.1610	0.3447	0.4013
38	2	1	0.6	µg/g f	0.0135	NA	0.0081	0.0961	0.5568	0.2098	0.1726	0.0843	0.0145	0.0386	0.0469
38	2	2	6	µg/g f	0.0135	NA	0.081	0.0961	0.5568	0.2098	0.1726	0.8429	0.1455	0.3861	0.4693
39	1	1	0.8	mg/kg/d	554	NA	0.4432	20	2.1147	7.3681	9.4574	0.0222	0.2096	0.0602	0.0469
39	1	2	6.4	mg/kg/d	554	NA	3.5456	20	2.1147	7.3681	9.4574	0.1773	1.6766	0.4812	0.3749
40	1	1	0.4	g/kg f	0.016	NA	0.0064	0.1326	0.6034	0.2600	0.2197	0.0483	0.0106	0.0246	0.0291
40	1	2	5.2	g/kg f	0.016	NA	0.0832	0.1326	0.6034	0.2600	0.2197	0.6275	0.1379	0.3200	0.3786
41	1	1	0.15	mg/kg f	0.021	NA	0.00315	0.223	0.6872	0.3677	0.3245	0.0141	0.0046	0.0086	0.0097
41	1	2	10	mg/kg f	0.021	NA	0.21	0.223	0.6872	0.3677	0.3245	0.9417	0.3056	0.5711	0.6471
44	1	1	1	mg/kg f	0.019	NA	0.019	0.179	0.6504	0.3176	0.2752	0.1061	0.0292	0.0598	0.0690
44	1	2	6	mg/kg f	0.019	NA	0.114	0.179	0.6504	0.3176	0.2752	0.6369	0.1753	0.3589	0.4143
45	1	1	6.2	µmol/kg f	0.017	NA	0.0067	0.161	0.6334	0.2959	0.2542	0.0416	0.0106	0.0226	0.0264
45	1	2	92.4	µmol/kg f	0.017	NA	0.1	0.161	0.6334	0.2959	0.2542	0.6211	0.1579	0.3379	0.3934
47	1	1	0.6	µg/g f	0.015	NA	0.009	0.1265	0.5964	0.2520	0.2121	0.0711	0.0151	0.0357	0.0424
47	1	2	6	µg/g f	0.015	NA	0.09	0.1265	0.5964	0.2520	0.2121	0.7115	0.1509	0.3571	0.4243
50	1	1	0.44	ppm	0.00407	NA	0.0018	0.0271	0.4057	0.0902	0.0668	0.0661	0.0044	0.0198	0.0268
50	1	2	4.98	ppm	0.00407	NA	0.0203	0.0271	0.4057	0.0902	0.0668	0.7479	0.0500	0.2247	0.3035
51	1	1	0.6	mg/kg f	0.018	NA	0.0108	0.304	0.7425	0.4521	0.4094	0.0355	0.0145	0.0239	0.0264
51	1	2	6	mg/kg f	0.018	NA	0.108	0.304	0.7425	0.4521	0.4094	0.3553	0.1454	0.2389	0.2638
52	1	1	0.4	mg/kg f	10.1	NA	0.004	0.271	0.7215	0.4188	0.3756	0.0148	0.0055	0.0096	0.0106
52	1	2	5.7	mg/kg f	10.1	NA	0.053	0.271	0.7215	0.4188	0.3756	0.1956	0.0735	0.1266	0.1411
52	2	1	0.4	mg/kg f	13.7	NA	0.0055	0.325	0.7550	0.4727	0.4304	0.0169	0.0073	0.0116	0.0128
52	2	2	5.7	mg/kg f	13.7	NA	0.089	0.325	0.7550	0.4727	0.4304	0.2738	0.1179	0.1883	0.2068

53	1	1	0	mg/L	NA	NA	0.007	0.108	0.6412	0.3057	0.2636	0.0415	0.0109	0.0230	0.0266
53	1	2	40	mg/L	NA	0.0151	0.604	0.108	0.6412	0.3057	0.2636	1.4414	0.3799	0.7969	0.9242
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
55	1	1	0.6	ppm	0.02	NA	0.012	0.358	0.7735	0.5042	0.4628	0.0335	0.0155	0.0238	0.0259
55	1	2	25	ppm	0.02	NA	0.5	0.358	0.7735	0.5042	0.4628	1.3966	0.6464	0.9917	1.0803
58	1	1	0.6	mg/kg f	0.0034	NA	0.00204	0.02276	0.3884	0.0803	0.0586	0.0896	0.0053	0.0254	0.0348
58	1	2	2	mg/kg f	0.0034	NA	0.0068	0.02276	0.3884	0.0803	0.0586	0.2988	0.0175	0.0847	0.1160
58	1	3	6	mg/kg f	0.0034	NA	0.0204	0.02276	0.3884	0.0803	0.0586	0.8963	0.0525	0.2540	0.3481
62	1	1	2.8	mg/kg f	0.018	NA	0.0504	0.315	0.7492	0.4630	0.4205	0.1600	0.0673	0.1089	0.1199
62	1	2	6.6	mg/kg f	0.018	NA	0.1188	0.315	0.7492	0.4630	0.4205	0.3771	0.1586	0.2566	0.2825
66	1	1	0.2	µg/g f	0.018	NA	0.0036	0.161	0.6334	0.2959	0.2542	0.0224	0.0057	0.0122	0.0142
66	1	2	1	µg/g f	0.018	NA	0.018	0.161	0.6334	0.2959	0.2542	0.1118	0.0284	0.0608	0.0708
66	1	3	2	µg/g f	0.018	NA	0.036	0.161	0.6334	0.2959	0.2542	0.2236	0.0568	0.1216	0.1416
66	1	4	3	µg/g f	0.018	NA	0.054	0.161	0.6334	0.2959	0.2542	0.3354	0.0852	0.1825	0.2125
66	1	5	4	µg/g f	0.018	NA	0.072	0.161	0.6334	0.2959	0.2542	0.4472	0.1137	0.2433	0.2833
67	1	1	0.35	mg/kg f	0.003	NA	0.00105	0.02	0.3761	0.0737	0.0532	0.0525	0.0028	0.0143	0.0197
67	1	2	6	mg/kg f	0.003	NA	0.018	0.02	0.3761	0.0737	0.0532	0.9000	0.0479	0.2443	0.3385
68	1	1	1.05	mg/kg f	0.002	NA	0.0021	0.01675	0.3598	0.0655	0.0466	0.1254	0.0058	0.0321	0.0451
68	1	2	6.4	mg/kg f	0.002	NA	0.0128	0.01675	0.3598	0.0655	0.0466	0.7642	0.0356	0.1955	0.2749
70	1	1	0.79	mg/kg	0.017	0.02254	0.01343	0.161	0.6334	0.2959	0.2542	0.0834	0.0212	0.0454	0.0528
70	1	2	3.79	mg/kg	0.017	0.02254	0.1094	0.161	0.6334	0.2959	0.2542	0.6795	0.6795	0.3697	0.4304
71	1	1	0.78	mg/d	NA	NA	0.78	70	2.8925	16.9850	24.2005	0.0111	0.2697	0.0459	0.0322
72	1	1	0.79	µg/ml	0.016	18.62	0.01264	0.133	0.6039	0.2606	0.2202	0.0950	0.0209	0.0485	0.0574
72	1	2	3.79	µg/ml	0.016	18.62	0.0706	0.133	0.6039	0.2606	0.2202	0.5308	0.1169	0.2710	0.3206

73	1	1	0.79	µg/ml	0.016	18.62	0.01264	0.133	0.6039	0.2606	0.2202	0.0950	0.0209	0.0485	0.0574
73	1	2	3.79	µg/ml	0.016	18.62	0.0706	0.133	0.6039	0.2606	0.2202	0.5308	0.1169	0.2710	0.3206
74	1	1	0.79	µg/ml	0.016	18.62	0.01264	0.133	0.6039	0.2606	0.2202	0.0950	0.0209	0.0485	0.0574
74	1	2	3.79	µg/ml	0.016	18.62	0.0706	0.133	0.6039	0.2606	0.2202	0.5308	0.1169	0.2710	0.3206
76	1	1	0.3	mg/kg f	0.0032	NA	0.00096	0.0221	0.3856	0.0788	0.0573	0.0434	0.0025	0.0122	0.0167
76	1	2	8.4	mg/kg f	0.0032	NA	0.02688	0.0221	0.3856	0.0788	0.0573	1.2163	0.0697	0.3413	0.4690
77	1	1	1	mg/kg f	0.019	NA	0.019	0.249	0.7064	0.3958	0.3525	0.0763	0.0269	0.0480	0.0539
77	1	2	7	mg/kg f	0.019	NA	0.133	0.249	0.7064	0.3958	0.3525	0.5341	0.1883	0.3360	0.3773
78	1	1	2.7	mg/kg f	0.02	NA	0.054	0.258	0.7127	0.4053	0.3620	0.2093	0.0758	0.1332	0.1492
78	1	2	6.2	mg/kg f	0.02	NA	0.124	0.258	0.7127	0.4053	0.3620	0.4806	0.1740	0.3060	0.3425
81	1	1	0.8	mg/kg f	0.014	NA	0.0112	0.0985	0.5602	0.2133	0.1758	0.1137	0.0200	0.0525	0.0637
81	1	2	1.7	mg/kg f	0.014	NA	0.0238	0.0985	0.5602	0.2133	0.1758	0.2416	0.0425	0.1116	0.1354
81	1	3	6.7	mg/kg f	0.014	NA	0.0938	0.0985	0.5602	0.2133	0.1758	0.9523	0.1674	0.4398	0.5335
83	1	1	7.9	µmol/kg f	0.015	NA	0.00753	0.116	0.5836	0.2379	0.0256	0.0649	0.0129	0.0317	0.2946
83	1	2	125.9	µmol/kg f	0.015	NA	0.12	0.116	0.5836	0.2379	0.2039	1.0345	0.2056	0.5045	0.5886
84	1	1	20	mg/L	0.018	19.18	0.39152	0.137	0.6084	0.2658	0.2252	2.8578	0.6435	1.4732	1.7387
84	1	2	0	mg/L	0.018	NA	0.00792	0.137	0.6084	0.2658	0.2252	0.0578	0.0130	0.0298	0.0352
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
85	1	1	0	ppm	0.02	32.62	0.008	0.233	0.6948	0.3786	0.3354	0.0343	0.0115	0.0211	0.0239
85	1	2	20	ppm	0.02	32.62	0.6604	0.233	0.6948	0.3786	0.3354	2.8343	0.9505	1.7441	1.9692
Purified base diet contains 0.4 mg/kg															
86	1	1	0.4	mg/kg/d	0.009	NA	0.0036	0.05753	0.4897	0.1490	0.1175	0.0626	0.0074	0.0242	0.0306
86	1	2	4	mg/kg/d	0.009	NA	0.036	0.05753	0.4897	0.1490	0.1175	0.6258	0.0735	0.2416	0.3065
89	1	1	0.6	mg/kg f	0.019	NA	0.0114	0.181	0.6523	0.3200	0.2775	0.0630	0.0175	0.0356	0.0411

89	1	2	7.5	mg/kg f	0.019	NA	0.1425	0.181	0.6523	0.3200	0.2775	0.7873	0.2185	0.4453	0.5135
90	1	1	0	mg/kg f	0.02	NA	0.005	0.268	0.7195	0.4157	0.3725	0.0201	0.0075	0.0130	0.0145
90	1	2	1.5	mg/kg f	0.02	NA	0.03	0.268	0.7195	0.4157	0.3725	0.1119	0.0417	0.0722	0.0805
90	1	3	3	mg/kg f	0.02	NA	0.06	0.268	0.7195	0.4157	0.3725	0.2239	0.0834	0.1443	0.1611
90	1	4	4.5	mg/kg f	0.02	NA	0.09	0.268	0.7195	0.4157	0.3725	0.3358	0.1251	0.2165	0.2416
90	1	5	6	mg/kg f	0.02	NA	0.12	0.268	0.7195	0.4157	0.3725	0.4478	0.1668	0.2887	0.3222
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
91	1	1	0.37	mg/kg	0.019	NA	0.00703	0.1807	0.6520	0.3196	0.2772	0.0389	0.0108	0.0220	0.0254
91	1	2	0.79	mg/kg	0.019	NA	0.01501	0.1807	0.6520	0.3196	0.2772	0.0831	0.0230	0.0470	0.0542
91	1	3	2.45	mg/kg	0.019	NA	0.04655	0.1807	0.6520	0.3196	0.2772	0.2576	0.0714	0.1456	0.1680
91	1	4	7.28	mg/kg	0.019	NA	0.13832	0.1807	0.6520	0.3196	0.2772	0.7655	0.2122	0.4328	0.4991
92	1	1	0.1	mg/kg f	0.018	NA	0.0018	0.199	0.6679	0.3409	0.2979	0.0090	0.0027	0.0053	0.0060
92	1	2	10	mg/kg f	0.018	NA	0.18	0.199	0.6679	0.3409	0.2979	0.9045	0.2695	0.5281	0.6041
95	1	1	9.4	µmol/kg f	0.015	NA	0.009	0.122	0.5910	0.2460	0.2064	0.0738	0.0152	0.0366	0.0436
95	1	2	103.9	µmol/kg f	0.015	NA	0.1	0.122	0.5910	0.2460	0.2064	0.8197	0.1692	0.4065	0.4844
96	1	1	1.3	mg/kg f	0.02	NA	0.026	0.377	0.7836	0.5219	0.4811	0.0690	0.0332	0.0498	0.0540
96	1	2	2.8	mg/kg f	0.02	NA	0.056	0.377	0.7836	0.5219	0.4811	0.1485	0.0715	0.1073	0.1164
96	1	3	6.7	mg/kg f	0.02	NA	0.134	0.377	0.7836	0.5219	0.4811	0.3554	0.1710	0.2568	0.2785
100	1	1	0.6	mg/kg f	0.019	NA	0.0114	0.181	0.6523	0.3200	0.2775	0.0630	0.0175	0.0356	0.0411
100	1	2	7.5	mg/kg f	0.019	NA	0.1425	0.181	0.6523	0.3200	0.2775	0.7873	0.2185	0.4453	0.5135
102	1	1	0.57	mg/1000kcal	NA	NA	1.6245	70	2.8925	16.9850	24.2005	0.0124	0.3008	0.0512	0.0359
102	1	2	1.61	mg/100kcal	NA	NA	4.5885	70	2.8925	16.9850	24.2005	0.0197	0.4771	0.0812	0.0570
106	1	1	0.57	mg/kg f	0.02	NA	0.0114	0.381	0.7857	0.5256	0.4849	0.0299	0.0145	0.0217	0.0235
106	1	2	5	mg/kg f	0.02	NA	0.1	0.381	0.7857	0.5256	0.4849	0.2625	0.1273	0.1903	0.2062

107	1	1	0.06	mg/kg	0.019	NA	0.00114	0.175	0.6468	0.3129	0.2706	0.0065	0.0018	0.0036	0.0042
107	1	2	20.03	mg/kg	0.019	NA	0.38114	0.175	0.6468	0.3129	0.2706	2.1779	0.5893	1.2182	1.4087
108	1	1	5	mg/kg	0.02	NA	0.1	0.25	0.7071	0.3969	0.3536	0.4	0.1414	0.2520	0.2828
108	1	2	2.5	mg/kg	0.02	NA	0.05	0.25	0.7071	0.3969	0.3536	0.2	0.0707	0.1260	0.1414
108	1	3	0.75	mg/kg	0.02	NA	0.015	0.25	0.7071	0.3969	0.3536	0.06	0.0212	0.0378	0.0424
115	1	1	6	ppm	0.0041	NA	0.0243	0.027	0.4054	0.09	0.0666	0.9	0.0599	0.27	0.3648
115	1	2	0.5	ppm	0.0041	NA	0.0020	0.027	0.4054	0.09	0.0666	0.075	0.0050	0.0225	0.0304
118	1	1	6.19	mg/kg	0.017	NA	0.10523	0.12	0.5886	0.2433	0.2039	0.8769	0.1788	0.4325	0.5161
118	1	2	0.43	mg/kg	0.017	NA	0.00731	0.12	0.5886	0.2433	0.2039	0.0609	0.0124	0.0300	0.0359
119	1	1	6	mg/kg	0.0178	NA	0.1068	0.12	0.5886	0.2433	0.2039	0.8900	0.1815	0.4390	0.5238
119	1	2	0.5	mg/kg	0.0178	NA	0.0089	0.12	0.5886	0.2433	0.2039	0.0742	0.0151	0.0366	0.0437
120	1	1	5.6	mg/kg	0.017	NA	0.0952	0.125	0.5946	0.25	0.2102	0.7616	0.7616	0.3808	0.4529
120	1	2	0.66	mg/kg	0.017	NA	0.01122	0.125	0.5946	0.25	0.2102	0.08976	0.08976	0.04488	0.0534
121	1	1	5.3	µg/g	0.013	NA	0.0689	0.085	0.5400	0.1933	0.1574	0.8106	0.1276	0.3564	0.4377
121	1	2	0.8	µg/g	0.013	NA	0.0104	0.085	0.5400	0.1933	0.1574	0.1224	0.0193	0.0538	0.0661
122	1	1	2.59	mg/d	NA	NA	2.59	87	3.0541	19.6342	28.4865	0.0298	0.8480	0.1319	0.0909
122	1	2	0.59	mg/d	NA	NA	0.59	87	3.0541	19.6342	28.4865	0.0068	0.1932	0.0300	0.0207
123	1	1	6	mg/kg	0.017	NA	0.102	0.13	0.6005	0.2566	0.2165	0.7846	0.1699	0.3975	0.4711
123	1	2	0.5	mg/kg	0.017	NA	0.0085	0.13	0.6005	0.2566	0.2165	0.0654	0.0142	0.0331	0.0393
125	1	1	5.88	mg/kg	0.02	NA	0.1176	0.44	0.8144	0.5785	0.5402	0.2673	0.1444	0.2033	0.2177
125	1	2	2.94	mg/kg	0.02	NA	0.0588	0.44	0.8144	0.5785	0.5402	0.1336	0.0722	0.1016	0.1088
125	1	3	1.62	mg/kg	0.02	NA	0.0324	0.44	0.8144	0.5785	0.5402	0.0736	0.0398	0.0560	0.0600

129	1	1	6	mg/d	NA	NA	6	78.4	2.9756	18.3180	26.3474	0.0765	2.0164	0.3275	0.2277
129	1	2	1.6	mg/d	NA	NA	1.6	78.4	2.9756	18.3180	26.3474	0.0204	0.5377	0.0873	0.0607
129	1	3	0.7	mg/d	NA	NA	0.7	78.4	2.9756	18.3180	26.3474	0.0089	0.2352	0.0382	0.0266
133	1	1	6	mg/kg	0.018	NA	0.108	0.167	0.6393	0.3033	0.2612	0.6467	0.1689	0.3561	0.4134
133	1	2	3	mg/kg	0.018	NA	0.054	0.167	0.6393	0.3033	0.2612	0.3234	0.0845	0.1781	0.2067
133	1	3	2.5	mg/kg	0.018	NA	0.045	0.167	0.6393	0.3033	0.2612	0.2695	0.0704	0.1484	0.1723
133	1	4	2	mg/kg	0.018	NA	0.036	0.167	0.6393	0.3033	0.2612	0.2156	0.0563	0.1187	0.1378
133	1	5	1.5	mg/kg	0.018	NA	0.027	0.167	0.6393	0.3033	0.2612	0.1617	0.0422	0.0890	0.1034
133	1	6	1	mg/kg	0.018	NA	0.018	0.167	0.6393	0.3033	0.2612	0.1078	0.0282	0.0594	0.0689
133	1	7	0.63	mg/kg	0.018	NA	0.01134	0.167	0.6393	0.3033	0.2612	0.0679	0.0177	0.0374	0.0434
133	2	1	6	mg/kg	0.015	NA	0.09	0.155	0.6275	0.2886	0.2470	0.5806	0.1434	0.3119	0.3643
133	2	2	3	mg/kg	0.015	NA	0.045	0.155	0.6275	0.2886	0.2470	0.2903	0.0717	0.1560	0.1822
133	2	3	2.5	mg/kg	0.015	NA	0.0375	0.155	0.6275	0.2886	0.2470	0.2419	0.0598	0.1300	0.1518
133	2	4	2	mg/kg	0.015	NA	0.03	0.155	0.6275	0.2886	0.2470	0.1935	0.0478	0.1040	0.1214
133	2	5	1.5	mg/kg	0.015	NA	0.0225	0.155	0.6275	0.2886	0.2470	0.1452	0.0359	0.0780	0.0911
133	2	6	1	mg/kg	0.015	NA	0.015	0.155	0.6275	0.2886	0.2470	0.0968	0.0239	0.0520	0.0607
133	2	7	0.63	mg/kg	0.015	NA	0.00945	0.155	0.6275	0.2886	0.2470	0.0610	0.0151	0.0327	0.0383
137	1	1	5.7	mg/kg	0.022	NA	0.1254	0.5	0.8409	0.6300	0.5946	0.2508	0.1491	0.1991	0.2109
137	1	2	3.1	mg/kg	0.022	NA	0.0682	0.5	0.8409	0.6300	0.5946	0.1364	0.0811	0.1083	0.1147
137	1	3	1.65	mg/kg	0.022	NA	0.0356	0.5	0.8409	0.6300	0.5946	0.0712	0.0423	0.0565	0.0599
141	1	1	20	mg/L	0.02	37.5	0.708	0.25	0.7071	0.3969	0.3536	2.832	1.0013	1.7840	2.0025
141	1	2	0	mg/L	0.02	37.5	0.008	0.25	0.7071	0.3969	0.3536	0.032	0.0113	0.0202	0.0226
141	2	1	20	mg/L	3	3	0.0612	0.02	0.3761	0.0737	0.0532	3.06	0.1627	0.8306	1.1507
141	2	2	0	mg/L	3	3	0.0012	0.02	0.3761	0.0737	0.0532	0.06	0.0032	0.0163	0.0226
Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).															
142	1	1	7.28	mg/kg	0.016	NA	0.1165	0.125	0.5946	0.25	0.2102	0.932	0.1959	0.466	0.5542
142	1	2	2.45	mg/kg	0.016	NA	0.0392	0.125	0.5946	0.25	0.2102	0.3136	0.0659	0.1568	0.1865
142	1	3	0.79	mg/kg	0.016	NA	0.0126	0.125	0.5946	0.25	0.2102	0.1008	0.0212	0.0504	0.0599

142	1	4	0.37	mg/kg	0.016	NA	0.0059	0.125	0.5946	0.25	0.2102	0.0472	0.0099	0.0236	0.0281
143	1	1	6	mg/kg	0.016	NA	0.096	0.125	0.5946	0.25	0.2102	0.768	0.1615	0.384	0.4567
143	1	2	0.3	mg/kg	0.016	NA	0.0048	0.125	0.5946	0.25	0.2102	0.0384	0.0081	0.0192	0.0228
144	1	1	6.18	mg/kg	0.017	NA	0.1051	0.125	0.5946	0.25	0.2102	0.8408	0.1768	0.4204	0.4999
144	1	2	0.29	mg/kg	0.017	NA	0.0049	0.125	0.5946	0.25	0.2102	0.0392	0.0082	0.0196	0.0233
148	1	1	10.5	mg/kg	0.021	NA	0.2205	0.232	0.6940	0.3776	0.3343	0.9504	0.3177	0.584	0.6596
148	1	2	0.43	mg/kg	0.021	NA	0.009	0.232	0.6940	0.3776	0.3343	0.0388	0.0130	0.024	0.0269
149	1	1	6.26	mg/kg	0.017	NA	0.1064	0.155	0.6275	0.2886	0.2470	0.6865	0.1696	0.369	0.4307
149	1	2	0.16	mg/kg	0.017	NA	0.0027	0.155	0.6275	0.2886	0.2470	0.0174	0.0043	0.009	0.0109
167	1	1	7.19	mg/kg	0.015	NA	0.1079	0.1	0.5623	0.2154	0.1778	1.0790	0.1919	0.501	0.6068
167	1	2	0.78	mg/kg	0.015	NA	0.0117	0.1	0.5623	0.2154	0.1778	0.1170	0.0208	0.054	0.0658
177	1	1	5.6	mg/kg	0.017	NA	0.0952	0.125	0.5946	0.2500	0.2102	0.7616	0.1601	0.381	0.4529
177	1	2	0.33	mg/kg	0.017	NA	0.00528	0.125	0.5946	0.2500	0.2102	0.0422	0.0089	0.021	0.0251
179	1	1	6.18	mg/kg	0.016	NA	0.0989	0.125	0.5946	0.2500	0.2102	0.7912	0.1663	0.396	0.4705
179	1	2	0.29	mg/kg	0.016	NA	0.0046	0.125	0.5946	0.2500	0.2102	0.0368	0.0077	0.018	0.0219
183	1	1	5.4	mg/kg	0.016	NA	0.0864	0.125	0.5946	0.2500	0.2102	0.6912	0.1453	0.346	0.4110
183	1	2	0.3	mg/kg	0.016	NA	0.0048	0.125	0.5946	0.2500	0.2102	0.0384	0.0081	0.019	0.0228
185	1	1	6	mg/kg	0.017	NA	0.1071	0.152	0.6244	0.2848	0.2434	0.7046	0.1715	0.376	0.4400
185	1	2	0	mg/kg	0.017	NA	0.0051	0.152	0.6244	0.2848	0.2434	0.0336	0.0082	0.018	0.0210

Background levels of copper in drinking water assumed to be 0.01 mg/L (Araya, 2004).

202	1	1	5	mg/kg	0.017	NA	0.085	0.125	0.5946	0.2500	0.2102	0.6800	0.1430	0.340	0.4043
202	1	2	0.25	mg/kg	0.017	NA	0.004	0.125	0.5946	0.2500	0.2102	0.0320	0.0067	0.016	0.0190

202	2	1	5	mg/kg	0.016	NA	0.075	0.115	0.5823	0.2365	0.1975	0.6522	0.1288	0.317	0.3798
202	2	2	0.25	mg/kg	0.016	NA	0.00375	0.115	0.5823	0.2365	0.1975	0.0326	0.0064	0.016	0.0190
211	1	1	5.7	mg/kg	0.021	NA	0.1197	0.5	0.8409	0.6300	0.5946	0.2394	0.1423	0.1900	0.2013
211	1	2	1.1	mg/kg	0.021	NA	0.0231	0.5	0.8409	0.6300	0.5946	0.0462	0.0275	0.0367	0.0388

Note. Ref ID #, reference ID number (corresponds with references and ID number in Tables 8 and 9); Exp, experiment number within the publication; Grp, group number within experiment; Base, reported dose of copper; BW, body weight; Cu, copper; ppm, parts per million; mg/ml, milligrams of copper per milliliter of water; mg/d, milligrams of copper per day; mg/L, milligrams of copper per liter of water; mg/kg f, milligrams of copper per kilogram of feed; µg /g f, micrograms of copper per gram of feed; g/kg f, grams of copper per kilogram of feed; µmol/kg f, micromoles of copper per kilogram of feed; µg/ml, micrograms of copper per millimeter of water; mg/1000kcal, milligrams of copper per 1000 kilocalories.

APPENDIX F: COPPER DATABASE – DEFINED BY RESPONSES TO ELEVATED AND DEFICIENT COPPER INTAKE

Table F1: Responses Associated with Studies on Copper Excess

Reference	*Species*	*Copper species/ study type/ exposure route/duration/sex*	*Effects*	*Dose groups*	*Severity scores*	*Group Size*
Alissa et al., 2004	Rabbits	NS/ Subchronic / Dietary / 84 days / Males	Increased body weight, increased plasma copper, increase liver copper, increased liver zinc, increased zinc in aorta, increased liver enzyme activity,	3.7 mg/d 350 mg/d	0 3	8 8
Araya et al. 2003	Humans	$CuSO_4$ / Acute / Drinking water / 1 time dose / Both Males & Females	Increased antral area and nausea	0 mg/l 10 mg/l	0 4	30
Araya et al. 2003b	Humans	$CuSO_4$/ Acute / Drinking water / 1 dose / Females	Increased nausea	0 mg/l 2 mg/l 4 mg/l 6 mg/l 8 mg/l	0 0 0 4 4	269
Araya et al. 2003c	Humans	$CuSO_4$ / Subchronic / Drinking water / 60 days / Both Males & Females	GI symptoms	0.01 mg/l 2 mg/l 4 mg/l 6 mg/l	0 0 0 4	60
Araya et al. 2004	Humans	$CuSO_4$ / Subchronic / Drinking water / 60 days / Both Males & Females	GI symptoms	0.02 mg/l 2 mg/l 4 mg/l 6 mg/l	0 0 0 4	343 327 355 340
Armstrong et al. 2004	Pigs	$CuSO_4$ & CuCit / Dietary / 40 days / Both Males & Females	Improved growth rate and feed efficiency	10 ppm 135 ppm 260 ppm	0 3 3	66 66 66

				15 ppm	0	66
				46 ppm	0	66
				77 ppm	0	66
				140 ppm	3	66
Baker et al., 1999	Humans	$CuSO_4$ / Subacute / Capsule / 42 days	No effect of copper supplementation on biochemical markers of bone metabolism	0 mg/day	0	12
				3 mg/day	0	12
				6 mg/day	0	12
Baker et al., 1999	Humans	$CuSO_4$ / Subacute / Capsule / 42 days	No effect of copper supplementation on biochemical markers of bone metabolism	1.6 mg/day	0	11
				6.0 mg/day	0	11
Becaria et al., 2006	Mice	$CuSO_4$ / Subchronic / Drinking water / 84 days / Males	Increased serum TNF alpha levels, increased MDA levels in brain	0 ppm	0	6
				2 ppm	3	6
Cristofori et al., 1992	Rats	NS / Chronic / Dietary / 210 days / Males & Females	Increased liver, kidney and skeletal Cu and increased liver ALT.	5 ppm	0	10
				200 ppm	2	10
		NS / Chronic / Dietary / 350 days / Males & Females	Increased liver, kidney and skeletal Cu and increased serum ALP.	5 ppm	0	10
				200 ppm	2	10
Cromwell et al., 1989	Pigs	$CuSO_4$ / Subacute / Dietary / 28 days	Increased weight gain, decrease in feed intake/body weight gain ratio and increased liver copper burden.	0 ppm	0	8
				125 ppm	3	8
				250 ppm	3	8
		CuO / Subacute / Dietary / 28 days	No effects.	0 ppm	0	8
				125 ppm	0	8
				250 ppm	0	8
Cunnane et al., 1985	Rats	NS / Subchronic / Dietary / 84 days	Increase in body weight;	6 mg/kg/day	0	10

			decrease in liver and heart weight; and altered levels of blood lipids.	250 mg/kg/day	3	10
Davis 2002b	Pigs	NS / Subacute / Dietary / 10 days / Males & Females	Increased body weight	20 ppm 195 ppm	0 3	54 54
Fuentealba et al., 1989	Rats	NS / Subacute / Dietary / 7 days	Liver necrosis and increased liver Cu burden.	20 ppm 1500 ppm	0 4	4 4
		NS / Subacute / Dietary / 28 days	Liver necrosis and increased liver Cu burden.	20 ppm 1500 ppm	0 4	4 4
		NS / Subchronic / Dietary / 56 days	Liver necrosis and increased liver Cu burden.	20 ppm 1500 ppm	0 4	4 4
		NS / Subchronic / Dietary / 84 days	Liver necrosis and increased liver Cu burden.	20 ppm 1500 ppm	0 4	4 4
		NS/ Chronic / Dietary / 112 days	Liver necrosis and increased liver Cu burden.	20 ppm 1500 ppm	0 4	4 4
Fuentealba et al., 2000	Rats	$CuSO_4$/Chronic/Dietary/126 days	Increased liver Cu, decreased hepatic metallothein, altered levels of Alanine Amine Transferase and sorbitol dehydrogenase	0 ppm 1500 ppm	0 3	5 5
Giovanetti et al., 1998	Mice	$CuSO_4$ / Subacute / Dietary / 28 days	Altered levels of GSSG, gluthione content, Cu-Zn SOD, and Cu burden in the lungs. Altered lung weight. Altered liver copper burden and weight.	4.98 ppm 200 ppm	0 3	6 6
Goldschmith et al., 2005	Rats – A	$CuSO_4$ / Subacute / Drinking Water / 20 days / Males & Females	Increased brain copper, reduced synaptic sensibility and facilitation capacity (brain	0.12 mg/d 12.12 mg/d	0 4	23 23

			function)			
Gurel et al., 2007	Rats	NS / Subchronic / Drinking water / 60 days / Females	Increased liver, kidney and lung copper, increased MT levels in brain and kidney and increased apoptotic index in liver	0 mg/l 100 mg/l 400 mg/l	0 4 4	8 8 8
Gross et al., 1989	Rats	$Cu(CH_3COO)_2$ / Chronic / Drinking Water/ 252 days	Altered liver copper burden, levels of liver function parameters and liver function.	0% 0.0125%	0 3	9 9
Haywood et al., 1980	Rats	NS/Subacute/Dietary/7days	Increased liver and kidney Cu	0 ppm 2000 ppm	0 1	4 4
		NS/Subacute/Dietary/14days	Increased liver and kidney Cu, liver histopathology.	0 ppm 2000 ppm	0 4	4 4
		NS/Subacute/Dietary/21days	Increase liver and kidney Cu, liver and kidney histopathology	0 ppm 2000 ppm	0 4	4 4
		NS/Subchronic/Dietary/42days	Increase liver and kidney Cu, liver and kidney histopathology	0 ppm 2000 ppm	0 4	4 4
		NS/Subchronic/Dietary/63days	Increase liver and kidney Cu, liver and kidney histopathology	0 ppm 2000 ppm	0 4	4 4
		NS/Subchronic/Dietary/105days	Increase liver and kidney Cu, liver and kidney histopathology	0 ppm 2000 ppm	0 4	4 4
Haywood et al., 1985	Rats	NA / Subacute,/ Dietary / 7days	Necrobiotic changes in liver in highest dose group, increased liver copper burden.	10 mg/kg 3000 mg/kg 4000 mg/kg 5000 mg/kg	0 1 1 1	4 4 4 4

		6000 mg/kg	4	4
NA/Subacute/Dietary/14 days	Liver and kidney histopathology. Increased copper burden in liver and kidney.	10 mg/kg	0	4
		3000 mg/kg	4	4
		4000 mg/kg	4	4
		5000 mg/kg	4	4
		6000 mg/kg	4	4
NA/ Subacute/Dietary/21 days	Liver and kidney histopathology. Increase copper burden in liver and kidney.	10 mg/kg	0	4
		3000 mg/kg	4	4
		4000 mg/kg	4	4
		5000 mg/kg	4	4
		6000 mg/kg	4	4
NA/Subacute/Dietary/28 days	Liver and kidney histopathology. Increase copper burden in liver and kidney.	10 mg/kg	0	4
		3000 mg/kg	4	4
		4000 mg/kg	4	4
		5000 mg/kg	4	4
		6000 mg/kg	4	4
NA/Subchronic/Dietary/35 days	Kidney and liver histopathology, extensive necrosis in liver at 6000 mg.kg, increased liver and kidney copper.	10 mg/kg	0	4
		3000 mg/kg	4	4
		4000 mg/kg	4	4
		5000 mg/kg	4	4
		6000 mg/kg	5	4
NA/Subchronic/Dietary/42 days	Kidney and liver histopathology, chronic hepatitis at 6000 mg/kg. Increased liver and kidney copper.	10 mg/kg	0	4
		3000 mg/kg	4	4
		4000 mg/kg	4	4
		5000 mg/kg	4	4
		6000 mg/kg	5	4
NA/Chronic/Dietary/105 days	Kidney and liver histopathology, chronic hepatitis at 6000 mg/kg. Increased liver and kidney copper.	10 mg/kg	0	4
		3000 mg/kg	4	4
		4000 mg/kg	4	4
		5000 mg/kg	4	4
		6000 mg/kg	5	4

Hebert, 1993	Rats	$CuSO_4$/Subacute/Drinking Water/15 days/Males	Decreased body weight, decreased longevity at 3000 and 10000ppm.	0 ppm 300 ppm 1000 ppm 3000 ppm 10000 ppm 30000 ppm	0 0 0 3 6 6	5 5 5 5 5 5
	Mice	$CuSO_4$/Subacute/Drinking Water/15 days/Males	Altered all body tissue histopathology and body weight at three highest doses. Decreased longevity at 3000 and 10000 ppm.	0 ppm 300 ppm 1000 ppm 3000 ppm 10000 ppm 30000 ppm	0 0 0 4 6 6	5 5 5 5 5 5
	Rats	$CuSO_4$/Subacute/Drinking Water/15 days/Females	Decreased longevity at 10000 and 30000 ppm. Altered body weight at three highest doses.	0 ppm 300 ppm 1000 ppm 3000 ppm 10000 ppm 30000 ppm	0 0 0 3 6 6	5 5 5 5 5 5
	Mice	$CuSO_4$/Subacute/Drinking Water/15 days/Females	Decreased final body weight, decreased kidney, liver and altered tissue histopathology in three highest dose groups. Decreased longevity at 10000 and 30000 ppm. Altered brain and lung weights at 4 highest dose groups.	0 ppm 300 ppm 1000 ppm 3000 ppm 10000 ppm 30000 ppm	0 0 3 4 6 6	5 5 5 5 5 5
	Rats	$CuSO_4$/Subacute/Dietary/15 days / Males	Increased liver and kidney copper at highest dose group. Lower liver weight, inflammation of liver, depletion of cells in bone marrow at the 2 highest dose groups.	0 ppm 1000 ppm 2000 ppm 4000 ppm 8000 ppm 16000 ppm	0 0 0 0 4 4	5 5 5 5 5 5

Rats	$CuSO_4$/Subacute/Dietary/15 days / Females	Inflammation of liver and depletion of cells in bone marrow at the two highest dose groups. Significantly lower liver weight at the three highest dose groups.	0 ppm 1000 ppm 2000 ppm 4000 ppm 8000 ppm 16000 ppm	0 0 0 3 4 4	5 5 5 5 5 5
Mice	$CuSO_4$/Subacute/Dietary/15 days / Males	Increased brain weights at the two highest dose groups. Increased liver weights and forestomach hyperplasia at the three highest dose groups.	0 ppm 1000 ppm 2000 ppm 4000 ppm 8000 ppm 16000 ppm	0 0 0 4 4 4	5 5 5 5 5 5
Mice	$CuSO_4$/Subacute/Dietary/15 days / Females	Increased brain and liver weights at the highest dose groups. Forestomach hyperplasia at the three highest dose groups.	0 ppm 1000 ppm 2000 ppm 4000 ppm 8000 ppm 16000 ppm	0 0 0 4 4 4	5 5 5 5 5 5
Rats	$CuSO_4$/Subchronic/Dietary/92 days / Males	Altered hematrocrit, hemoglobin, at the 2 highest doses. Altered mean cell volume and hemoglobin at the 3 highest doses. Altered erythrocytes, reticulocytes at the highest dose group.	0 ppm 500 ppm 1000 ppm 2000 ppm 4000 ppm 8000 ppm	0 1 3 4 4 4	5 5 5 5 5 5
Rats	$CuSO_4$/Subchronic/Dietary/92 days / Females	Altered hematocrit and hemoglobin at highest dose group. Altered mean cell volume and hemoglobin at the three highest dose groups.	0 ppm 500 ppm 1000 ppm 2000 ppm 4000 ppm 8000 ppm	0 1 3 4 4 4	5 5 5 5 5 5
Mice	$CuSO_4$/Chronic/Dietary/92 days /	Altered heart and kidney	0 ppm	0	5

		Males	weights at highest dose group. Altered liver weight and forestomach hyperplasia at three highest dose groups.	1000 ppm 2000 ppm 4000 ppm 8000 ppm 16000 ppm	0 0 4 4 4	5 5 5 5 5
	Mice	$CuSO_4$/Chronic/Dietary/92 days / Females	Altered kidney and thymus weights at the highest dose group. Altered liver weight at the two highest dose groups. Forestomach hyperplasia at the three highest dose groups.	0 ppm 1000 ppm 2000 ppm 4000 ppm 8000 ppm 16000 ppm	0 0 0 4 4 4	5 5 5 5 5 5
Jantsch et al., 1985	Humans	$CuSO_4$ / Acute / Dietary / 1 dose	Hepatoxicity	250 g/day	4	1
Jones et al., 1997	Humans	Copper as glycine-chelate / Subacute / Capsule / 28 days	No effect on blood copper enzyme activities and indicators of cardiovascular disease risk	0 mg/day 2 mg/day	0 0	20 20
Kvietkauskaite et al., 2004	Mice	$CuSO_4$ / Subchronic / Drinking water / 133 days / Males	Decreased body weight, decreased liver weight, increased liver copper, decreased protein in liver, increased cytosis SOD activity, increased liver catalase activity , decreased cells of the immune system	0 mg/kg bw/d 22 mg/kg bw/d 42 mg/kg bw/d	0 3 3	10 10 10
Lai et al., 2005	Rats	$CuSO_4$ / Subacute / Drinking water / 7 days / Males	Decreased calcium in femoral diaphyseal	0 µg/mL 50 µg/mL 100 µg/mL 200 µg/mL	0 0 0 3	6 6 6 6
Massie et al., 1984	Mice	$C_{12}H_{22}CuO_{14}$ / Chronic / Drinking Water / 906 and 776 / Males	Altered body weight. Altered serum, liver, kidney, brain and cardiovascular levels of copper. Decreased lifespan.	0 ppm 317 ppm	0 6	8 8

Mullins et al., 1998	Rats	$CuSO_4$ / Subchronic / Dietary / 42 days / Males	Altered levels of metallothionein in liver, duodenum and kidney.	10 mg/kg 1500 mg/kg	0 2	
Murthy et al., 1981	Rats	$CuSO_4$ / Subacute / Dietary / 30 days	Increase dopamine, norepinephrine, 5-hydroxytrptamine and Cu in brain	0 mg/day 5 mg/day	0 3	6 6
O'Connor et al., 2003	Humans	$CuSO_4$ & Cu Glycine Chelates / Subacute / Capsule / 42 days / Males & Females	Increased serum dioxides, increased WBC cytochrome oxidase	1.23 mg/day 4.23 mg/day 1.23 mg/day 4.23 mg/day 1.23 mg/day 7.23 mg/day	0 1 0 1 0 1	15 15 15 15 15 15
O'Donohue et al., 1993	Humans	NS / Chronic / Capsule / 1095 days / Male	Liver cirrhosis	45 mg/day	4	1
Ozcelik et al., 2002	Rats – A	$CuSO_4$ / Subchronic / Drinking Water / 54 days / Males & Females	Decreased erythrocyte deformability, decreased Hb, decreased erythrocyte count, increased serum copper, increased blood viscosity	0 µg / mL 250 µg/mL	0 3	7 7
Pizarro et al., 1999b	Humans	$CuSO_4$ / Subchronic / Drinking Water / 77 day / Females	Increased GI symptoms	0 mg/l 1 mg/l 3 mg/l 5 mg/l	0 0 4 4	60 60 60 60
Pratt et al., 1985	Humans	$C_{12}H_{22}CuO_{14}$ / Subchronic / Capsule / 84 days	Decrease in serum potassium	0 mg/day 10 mg/day	0 2	7 7
Rana et al., 1980	Rats	$CuSO_4$ / Subacute / Dietary / 20 days / Males	Altered liver weight, growth and body weight. Altered hemoglobin, hematocrit and	0 mg/kg/day 100mg/kg/day	0 4	10 10

			RBC count. Histopathological changes in the liver and kidney			
Turnlund et al., 2004	Humans	NS / Subacute / Capsule / 18 days / Males	Increased superoxide dismutase, increased Cp activity, increased Benzylamine oxidase activity, increased urinary copper, increased hair copper	7.8 mg/d	2	11
Zhang et al., 2000	Rats	$CuSO_4$ / Subchronic / Capsule / 40 days / Both Males & Females	Altered copper burden, serum ALT, liver SOD, RBC SOD activity, liver gluthione peroxidise activity, liver MDA and serum MDA	0 mg/kg/day 500 mg/kg/day	0 3	4 4

Table F2: Responses Associated with Studies on Copper Deficiency

Reference	*Species*	*Copper species/ study type/ exposure route/duration*	*Effects*	*Dose groups*	*Severity scores*	*Group Size*
Ajayi et al. 2005	Rats	$CuCO_3$ / Subchronic / Dietary / 42 days / Males	Impaired body weight, depigmentation of hair, reduced packed cell volume, reduced white blood cell count, altered metabolism as reflected by liver and kidney content of trace elements	0.06 mg/kg 20.03 mg/kg	3 0	5 5
Allen et al., 1978	Rats	$CuSO_4$ / Subchronic / Dietary / 63 days / Males	Altered levels of cholesterol, hematocrit, body weight, liver Cu burden, heart weight and heart weight to body weight ratio	0.57 µg 5 µg	3 0	10 10
Allen et al., 1988	Rats	NS / Subchronic / Dietary / 49 days / Males	Altered levels of renal, liver and arteriole GSH, liver SOD and liver GSH-PX	0.2 µg 10 µg	2 0	8 8
Allen et al., 1978	Rats	$CuSO_4$ / Chronic / Dietary / 168 days / Males	Altered cholesterol metabolism	0.57 µg/g 5 µg/g	2 0	10 10
Allen et al., 1996	Rats	$CuCO_3$ / Chronic / Dietary / 140 days / Males	Altered hematocrit, ceruloplasmin body weight, growth efficiency, growth potential index, liver weight, liver weight to body weight ratio, heart weight to body weight ratio, kidney weight to body weight ratio and Cu levels in the spleen, liver, kidney and cardiovascular system	5.79 mg/kg 0.46 mg/kg	0 3	5 5

Andersen et al., 2007	Rats	$CuSO_4$ / Subchronic / Dietary / 49 days / Females	Decreased serum copper and iron, decreased liver iron and copper	5 mg/kg 2.5 mg/kg 0.75 mg/kg	0 3 3	8 8 8
Arce et al., 1992	Mice	$CuSO_4$ / Subchronic / Dietary / 39 days / Females	Altered hematocrit, liver iron content and ceruloplasmin.	1 ppm 10 ppm	2 0	21 21
Auclair et al., 2006	Mice	$CuCO_3$ / Subchronic / Dietary / 84 days / Males	Decreased copper concentration, decreased plasma Cp activity, decreased hematocrit, decreased hemoglobin, altered mean cell volume, decreased mean cellular hemoglobin concentration	6 ppm 0.5 ppm	0 3	16 16
Baker et al., 1999	Humans	$CuSO_4$ / Subchronic / Dietary / 42 days / Males	Altered levels of urinary pyridinoline and deoxypyridinoline	1.6 mg/day 0.7 mg/day	0 2	11 11
Bala et al., 1992	Pig	NS / Subchronic / Dietary / 77 days / Males & Females	Altered heart weight, serum and liver copper burden and mononuclear cell reactivity.	0.8 mg/kg/day 6.4 mg/kg/day	3 0	6 6
Bala et al., 1990	Rats	$CuCO_3$ / Subchronic / Dietary / 35 days/ Males $CuCO_3$ / Subchronic / Dietary / 56 days / Males	Altered levels of heart weight to body weight ratio, immune functioning indicators and mitogen reactivity	0.6 μg/g 6 μg/g 0.6 μg/g 6 μg/g	3 0 2 0	13 13 13 13
Bode et al., 1992	Rats	$CuSO_4$ / Subacute / Dietary / 28 days / Males	Altered body weight; hematocrit; Cu burden in skeletal muscle, kidney, liver and serum; heart weight, cholesterol, ceruloplasmin, respiration rate in liver and kidney and liver acceptor control index	0.4 g/kg 5.2 g/kg	3 0	16 16

Bremmer et al., 1987	Rats	$CuSO_4$ / Subchronic / Dietary / 42 days / Males	Altered liver and serum Cu burden; gluthione peroxidase in liver, kidney MT-I and body weight.	0.15 mg/kg/day 10 mg/kg/day	3 0	8 8
Chen et al., 2002	Rats	NS / Subchronic / Dietary / 35 days / Males	Decreased body weight and heart weight, decreased heart weight to body weight ratio, decreased liver SOD activity, altered mitochondrial respiration in presence of NADH	7.19 mg/kg 0.78 mg/kg	0 3	7 8
Cockell et al., 2005	Rats	NS / Subacute / Dietary / 30 days / Males	Decreased liver copper, decreased total SOD, decreased plasma copper, decreased plasma iron, decreased plasma carbonyl	6 mg/kg 0.5 mg/kg	0 3	30 30
Cunnane et al., 1985	Rats	NS / Subchronic / Dietary / 84 days / Males	Altered liver and body weight; liver weight to body weight ratio; heart weight to body weight ratio; plasma Cu; phospholipid fatty acid composition in the plasma, liver and heart; and liver triglyceride fatty acid composition.	1 mg/kg/day 6 mg/kg/day	3 0	10 10
Davidson et al., 1992	Rats	$CuCO_3$ / Subchronic / Dietary / 35 days / Males	Altered body weight, heart weight, heart weight to body weight ratio, liver SOD, hematocrit, cardiovascular histopathology, heart rate and EKG variables	6.2 µmol/kg 92.4 µmol/kg	3 0	6 6
Davis et al., 2002	Rats	NS / Subacute / Dietary / 28	Decreased cp activity,	5.3 µg/g	0	9

		days / Males	decreased copper concentration in plasma and liver	0.8 µg/g	2	9
Davis et al., 2003	Humans	$CuSO_4$ / Subacute / Dietary / 42 days / Males	Decreased fecal copper concentration, increased in vitro production of hydroxyl radicals, increased intestinal phosphatase activity in fecal water	2.59 mg/d 0.59 mg/d	0 3	17 17
DiSilvestro et al., 1992	Rats	$CuSO_4$ / Subchronic / Dietary / 42 days / Males	Altered levels of plasma caeruloplasmin and liver copper burden in the two highest dose groups. Altered body weight, heart weight to body weight ratio, hemoglobin and plasma cholesterol in highest dose group	8 ppm 2.5 ppm 0.2 ppm	0 2 3	16 16 16
Dong et al., 2005	Rats	$CuSO_4$ / Subchronic / Dietary / 35 days / Males	Decreased liver & kidney copper, increased heart weight, increased contractility, altered IGF levels, decreased hematocrit, increased liver iron	6 mg/kg 0.5 mg/kg	0 4	10 10
Falcone et al., 2005	Rats	$CuSO_4$ / Chronic / Dietary / 180 days / Males	Decreased kidney copper	5.88 mg/kg 2.94 mg/kg 1.62 mg/kg	0 0 1	9 9 8
Fields et al., 1997	Rats	NS / Subacute / Dietary / 28 days / Males & Females	Altered body weight; liver weight to body weight; pancreas weight; liver and pancreatic Cu burden; lipid peroxidation; amylase activity, lipase and insulin in the pancreas; and plasma insulin levels	0.6 µg/g 6 µg/g	3 0	10 10

Giovanetti et al., 1998	Mice	$CuSO_4$ / Subacute / Dietary / 28 days / Males	Altered body weight; liver and respiratory system Cu burden and levels of Cu-Zn SOD and GSSG in the respiratory system	0.44 ppm 4.98 ppm	3 0	36 36
Gitlin et al., 1992	Rats	NS / Subacute / Dietary / 28 days / Males and Females	Altered levels of ceruloplasmin and serum Cu	0.6 mg/kg/day 6 mg/kg/day	2 0	4 4
Gobejishvili et al., 2002	Rats	$CuSO_4$ / Subacute / Dietary / 28 days / Males	Decreased hematocrit, decreased copper in liver, Reduce time to 50% relaxation induced by L-arginine	5.6 mg/kg 0.33 mg/kg	0 4	21 21
Goodman et al., 1973	Rats	$CuSO_4$ / Subchronic / Drinking Water / 60 days / Males	Altered heart weight, heart weight to body weight ratio and cardiovascular histopathology.	0 mg/L 40 mg/L	3 0	4 6
Gordon et al., 2005	Rats	$CuSO_4$ / Subacute / Dietary / 28 day / Males	Decreased liver copper, altered mean fluorescent intensity in neutrophils, altered size and shape of neutrophils	6.18 mg/kg 0.29 mg/kg	0 3	4 4
Hamilton et al., 2000	Mice	NS / Subchronic / Dietary / 98 days / Males	At the two lowest dose groups, altered heart weight; liver and kidney weight; liver and kidney copper, enzyme activities in the liver and kidney; aortic lesions; cholesterol and triglyceride levels; and ceruloplasmin oxidase activity	0.6 mg/kg/day 2 mg/kg/day 6 mg/kg/day	4 4 0	12 12 11
Harvey et al., 2003	Humans	$CuCl_2$ / Subacute / Dietary / 56 days / Males	Decreased loss of endogenous copper stores	6 mg/d 1.6 mg/d 0.7 mg/d	0 1 1	12 12 12
Hopkins et al., 1995	Rats	$CuCO_3$ / Chronic / Dietary / 161 days / Males and Females	Altered serum Cu burden; hemoglobin; mitogen and mononuclear cell activity in the	2.8 mg/kg/day 6.6 mg/kg/day	3 0	5 5

			spleen and neutrophil activity			
Johnson et al., 1993	Rats	NS / Subchronic / Dietary / 35 days / Males	Altered ceruloplasmin activity; plasma Cu burden; RBC SOD activity; liver Cu burden; liver cytochrome C oxidase activity and platelet cytochrome-C oxidase activity in the four lowest dose groups. Altered levels of Cu-Zn SOD in liver in the three lowest dose groups. Altered red cell distribution width, hematocrit and hemoglobin in the two lowest dose groups	0.2 µg/g 1 µg/g 2 µg/g 3 µg/g 4 µg/g	3 3 2 2 0	10 10 10 10 10
Johnson et al., 2004	Rats	$CuSO_4$ / Subacute / Dietary / 35 days / Males	Decreased liver copper, decreased liver iron, decreased body weight, decreased hemoglobin & hematocrit, decreased amine oxidase, lower CCO activity in mitochondria, increased HO-1 content in liver, increased HO-1 in heart	5.4 mg/kg 0.3 mg/kg	0 3	10 10
Johnson et al., 2005	Rats	$CuSO_4$ / Subchronic / Dietary / 42 days / Males	Decreased liver & kidney copper, decreased liver CCO, decreased Cp, increased hemoglobin, decreased et heart weight, decreased SOD	6 mg/kg 3 mg/kg 2.5 mg/kg 2 mg/kg 1.5 mg/kg 1 mg/kg 0.63 mg/kg	0 1 1 1 2 2 3	7 7 7 7 7 7 7
Kang et al., 2000	Mice	NS / Subchronic / Dietary / 35 days / Males & Females	Altered Cu-Zn SOD; ceruloplasmin; heart weight; body weight; Cu burden in the liver and cardiovascular system; and gene expression in the	0.35 mg/kg/day 6 mg/kg/day	3 0	10 10

			cardiovascular system			
Karimbakas et al., 1988	Mice	NS / Subacute / Dietary / 21 days / Males	Altered body weight; heart weight; spleen weight; thymus weight; ceruloplasmin; Cu burden; hemoglobin; hematocrit; RBC Cu-Zn SOD activity; neutrophil count, lymphocyte count, lung Cu-Zn SOD, Ly-6g levels in immune system and respiratory myelo-peroxidase activity	1.05 µg/g 6.4 µg/g	3 0	6 6
Kelley et al., 1995	Human	NA / Subacute / Dietary / 24 days / Males	Altered levels of lymphocytes	0.66 mg/day	2	11
Klaahsen et al., 2007	Rats	$CuCO_3$ / Subchronic / Dietary / 35 days / Males	Decreased final heart weight, decreased body weight and heart weight to body weight ratio, decreased hematocrit, decrease CU SOD in liver	6 mg/kg 0 mg/kg	0 3	4 4
Klevay et al., 1981	Rats	$CuSO_4$ / Subchronic / Dietary / 35 days / Males	Altered EKG variables, plasma cholesterol and longevity	0.79 µg/g 3.79 µg/g	6 0	20 20
Klevay et al., 1985	Mice	$CuSO_4$ / Chronic / Drinking Water / 100 days / Female	Altered heart rate and Pr interval	0 µg/ml 10 µg/ml	3 0	16 16
Klevay et al., 1986	Humans	NA / Subchronic / Dietary / 150 days / Males	Altered plasma glucose, insulin and plasma Cu levels	0.78 mg/day	3	5
Lai et al., 1994	Rats	$CuSO_4$ / Subacute / Drinking Water / 28 days / Males	Altered levels of liver and cardiovascular SOD activity; liver and cardiovascular SOD protein; and liver mRNA	0 µg/ml 3 µg/ml	2 0	10 10
Lai et al., 1995	Humans	NA / Subchronic / Drinking Water / 28 days / Males	Altered levels of hematocrit, body weight, liver Cu burden,	0 µg/ml 3 µg/ml	3 0	10 10

			plasma cholesterol, liver enzyme activity, cardiovascular enzyme activity, liver enzyme mRNA and liver protein concentration			
Lai et al., 1996	Rats	$CuSO_4$ / Subacute / Drinking Water / 28 days / Males	Altered hematocrit, body weight, heart weight, liver weight, Cu burden in the liver and liver enzyme activity	0 µg/ml 3 µg/ml	3 0	10 10
Li et al., 2005	Rats	NS / Chronic / Dietary / 470 days / Males	Decreased kidney and liver copper, progressive deterioration of heart, swelling and disorganization of mitochondria in myocardium, altered ECG	5.7 mg/kg 3.1 mg/kg 1.65 mg/kg	0 4 4	5 5 5
Li et al., 2005	Rats	$CuSO_4$ / Subacute / Dietary / 35 days / Males	Decreased liver copper, decreased heart copper, decreased body weight, decreased heart weight, decreased hematocrit, decreased liver iron	7.28 mg/kg 2.45 mg/kg 0.79 mg/kg 0.37 mg/kg	0 3 3 3	4 8 7 4
Mao et al., 1998	Rats	NS / Subchronic / Dietary / 77 days / Males	Altered body weight, heart weight, hematocrit, liver Cu burden and cardiovascular histopathology	1 mg/kg/day 7 mg/kg/day	3 0	4 4
Mao et al., 1999	Rats	$CuCO_3$ / Subchronic / Dietary / 84 days / Males	Altered liver SOD activity, altered cardiovascular EKG variable	2.7 mg/kg/day 6.2 mg/kg/day	3 0	5 5
Menino et al., 1986	Mice	$CuCO_3$ / Subchronic / Dietary / 60 days / Females	Altered hemoglobin, hematocrit, and body weight at the three lowest doses. Altered in vitro blastocyte formation,	11 ppm 6 ppm 5 ppm 4 ppm	0 0 0 0	15 19 19 20

			fertilization rate and heart weight at the two lowest doses	3 ppm 2 ppm 1 ppm	3 4 4	20 19 18
Olin et al., 1994	Rats	NS / Subacute / Dietary / 21 days / Males & Females	Altered red cell Se-GSHPx activity, RBC SOD activity, extracellular SOD, SE-GSHPx activity, ceruloplasmin, thyroid hormone levels, anti-oxidant defense, plasma Cu burden, brain Cu burden and liver Cu burden	7.9 nmol/g 125.9 nmol/g	3 0	28 36
Prohaska et al., 1982	Rats	$CuSO_4$ / Subchronic / Drinking Water / 35 days / Males	Altered body weight, heart weight, hematocrit, ceruloplasmin, Cu burden, norepinephrine levels, left ventricular pressure, and oxygen consumption	0 ppm 20ppm	3 0	11 10
Prohaska et al., 1994	Rats	$CuSO_4$ / Subacute / Dietary / 28 days / Males and Females	Altered brain weight, body weight, hemoglobin, ceruloplasmin, liver Cu burden, brain Cu burden, norepinephrine levels, dopamine levels, brain DBM activity and adrenal DBM activity	0.4 mg/kg/day 4 mg/kg/day	3 0	17 17
Prohaska et al., 1995	Rats	$CuSO_4$ / Subchronic / Drinking Water / 38.5 days / Males	Altered heart weight, Cu burden in the brain and liver, body weight, and liver iron	0 mg/L 20 mg/L	3 0	4 4
Prohaska et al., 2001	Rats	$CuSO_4$ / Subacute / Drinking Water / 30 days / Females	Altered body weight, liver Cu burden, brain Cu burden, adrenal dopamine enzyme activity, brain dopamine enzyme activity, adrenal Cu-Zn	20 mg/l/day 0 mg/l/day	0 3	6 6

			SOD, and brain Cu, Zn SOD			
Prohaska et al., 2003	Mice & Rats	$CuSO_4$ / Subchronic / Drinking water / 35 days / Females	Decreased liver copper, decreased liver SOD, decreased hematocrit	20 mg/L 0 mg/L 20 mg/L 0 mg/L	0 2 0 3	5 5 5 5
Rayssiguier et al., 1993	Rats	$CuCO_3$ / Subchronic / Dietary / 42 days / Males	Altered body weight, liver weight, heart weight, hematocrit, plasma Cu burden, plasma triglycerides, plasma lipids, plasma APO B, serum lipid peroxidation, cardiovascular lipid peroxidation and liver lipid peroxidation	0.6 mg/kg/day 7.5 mg/kg/day	3 0	12 12
Reeves et al., 2005	Rats	NS / Subacute / Dietary / 19 days / Males NS/ Subacute / Dietary / 19 days / Females	In both males and females decreased serum copper, decreased serum iron, decreased serum Cp activity, decreased serum SOD activity, altered soleus muscle CO1 activity, decreased hemoglobin, decreased MCV, decreased RDW, decreased iron absorption, decreased iron excretion, decreased iron transporter protein, decreased liver, kidney, duodenal and serum copper at deficient dose	5.0 mg/kg 0.25 mg/kg 5.0 mg/kg 0.25 mg/kg	0 3 0 3	8 8 8 8
Rock et al., 1995	Rats	$CuCO_3$ / Subchronic / Dietary / 42 days / Males	Altered body weight, heart weight, hematocrit, RBC SOD activity, plasma Cu burden, total cholesterol, RBC survival, RBC half-life, fluorenscence	0.6 mg/kg/day 7.5 mg/kg/day	3 0	10 10

			anisotropy, RBC hemolysis and RBC TBARS			
Saari et al., 1999	Rats	NS / Subchronic / Dietary / 35 days / Males	Altered liver and heart Cu burden and heart weight at the three lowest doses. Altered hematocrit, haemoglobin, and cardiac resistance, heart rate and stroke volume at the highest dose	6 ppm 0.8 ppm 0.4 ppm 0 ppm	0 3 3 3	5 5 5 5
Saari et al., 2002 (91)	Rats	$CuSO_4$ / Subchronic / Dietary / 35 days / Males	Altered Cu levels in the cardiovascular system and the liver in the three lowest dose groups. Altered bodyweight, heart weight and hematocrit in the lowest dose group	0 mg/kg/day 1.6 mg/kg/day 3.2 mg/kg/day 24 mg/kg/day	3 1 1 0	4 7 8 4
Saari et al., 2002	Rats	$CuSO_4$ / Subchronic / Dietary / 35 days / Males	Altered platelet count in the four lowest dose groups. Altered RBC distribution width in the two lowest dose groups. Altered haemoglobin, heart weight, neutrophil count and haemoglobin in the lowest dose group	0.27 mg/kg/day 1.43 mg/kg/day 2.92 mg/kg/day 4.27 mg/kg/day 6.15 mg/kg/day	3 2 2 0 0	13 13 13 13 13
Saari et al., 2007	Rats	$CuSO_4$ / Subchronic / Dietary / 35 days / Males	Decreased liver copper & iron, decreased body and heart weight, decreased hematocrit, increased cardiac iNOS, altered eNOS protein levels, eleated total cardiac NOS activity	6 mg/kg 0.3 mg/kg	0 3	25 25
Schuschke et al., 1995	Rats	NS / Subacute / Dietary / 7 days / Males /	Altered bleeding time and liver copper burden.	6 ppm 3 ppm 1.5 ppm	0 1 3	5 5 5

		NS / Subacute / Dietary / 21 days / Males	Altered bleeding time and liver copper burden.	6 ppm 3 ppm 1.5 ppm	0 1 3	7 6 5
		NS / Subacute / Dietary 35 days / Males	Altered bleeding time and liver copper burden.	6 ppm 3 ppm 1.5 ppm	0 3 3	5 6 6
Schuschke et al., 1999	Rats	$CuSO_4$ / Subacute / Dietary / 28 days / Males	Altered erythrocyte Cu, Zn-SOD activity, aortic Cu, Zn-SOD activity, hematocrit and mean arterial pressure in the three lowest doses	0 ppm 1.5 ppm 3 ppm 6 ppm	3 3 3 0	6 5 6 5
Schuschke et al., 2002	Rats	$CuSO_4$ / Subacute / Dietary / 28 days / Males	Decreased body weight, decreased liver copper, decreased heart weight to body weight ratio, greater MPO activity in lung and kidney	6.18 mg/kg 0.29 mg/kg	0 3	5 5
Smith et al., 2002	Rats	NS / Subchronic / Dietary / 49 days / Males	Decreased Cp, decreased copper and iron in liver, bone mineral density loss in femur	5.7 mg/kg 1.1 mg/kg	0 3	18 18
Sugawara et al., 1999	Rats	$CuCl_2$ / Subchronic / Dietary / 35 days / Males & Females	Altered liver, kidney, GI Cu burden and liver metallothionein	0.5 mg/kg/day 10 mg/kg/day	2 0	5 5
Turnlund et al., 1990	Humans	$CuSO_4$ / Subchronic / Dietary / 90 days / Males	No effects	0.785 mg/day 1.68 mg/day	1 0	11 11
Wang et al., 1996	Rats	$CuCO_3$ / Subchronic / Dietary / 42 days / Males	Altered body weight, liver weight heart weight and liver u burden.	9.4 µmol/kg 103.9 µmol/kg	3 0	8 8
Welch et al., 2007	Rats	NS / Subchronic / Dietary / 60 days / Males	Decreased p-phenylenediamine oxidase activity in serum, decreased serum Cp, decreased	10.5 mg/kg 0.43 mg/kg	0 3	5 5

			body weight, decreased liver copper and iron, iron deposits in spleen			
Wildman et al., 1995	Rats	NS / Chronic / Dietary / 154 days / Males	Altered cardiovascular histopathology at the two lowest doses. Altered liver Cu burden, ceruloplasmin, serum Cu burden at the lowest dose	1.3 mg/kg/day 2.8 mg/kg/day 6.7 mg/kg/day	3 3 0	6 6 6
Zeng et al., 2007	Rats	$CuSO_4$ / Subchronic / Dietary / 35 days / Males	Decreased body weight, decreased heart to body weight ratio, decreased hemoglobin and hematocrit, decreased copper in heart and liver, perturbed mitochondrial function, decreased protein expression of COX I, Vb, V1b	6.26 mg/kg 0.16 mg/kg	0 3	10 10

APPENDIX G: ERC10 PLOTS FOR RATS AND MICE WITH THE CUMULATIVE ODDS MODEL FOR COPPER EXCESS

Weanling Rat Dietary Stratum

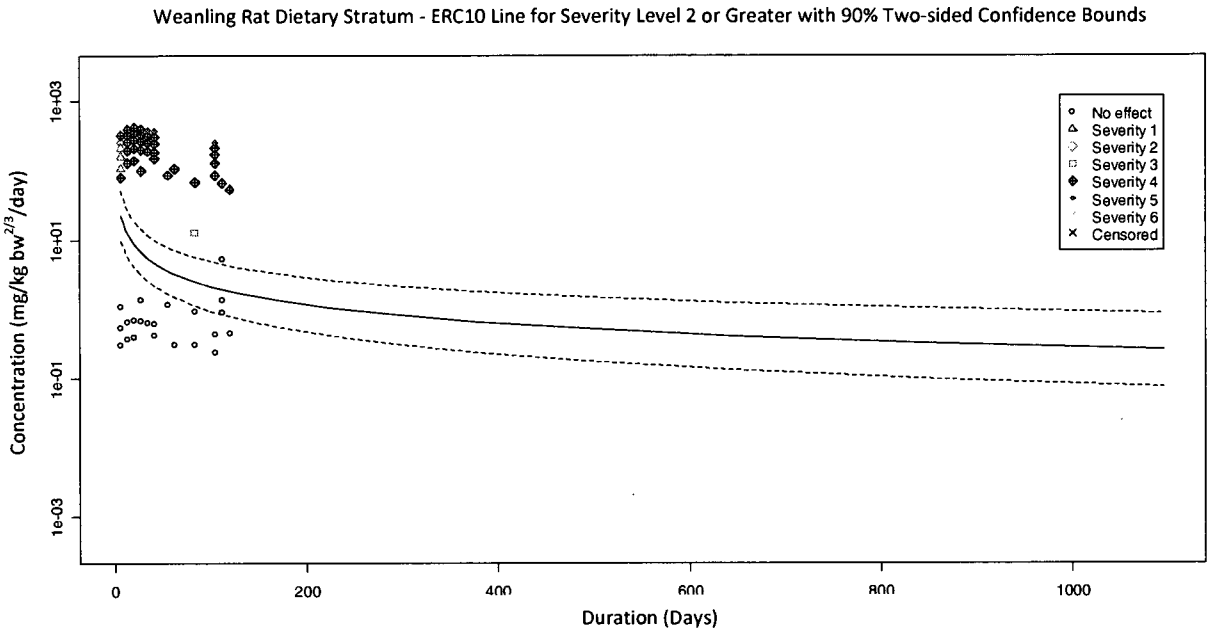

Figure G1: Cumulative odds model with the logit link function. Intercept stratified by the animal species and the route of exposure. Duration stratified by age.

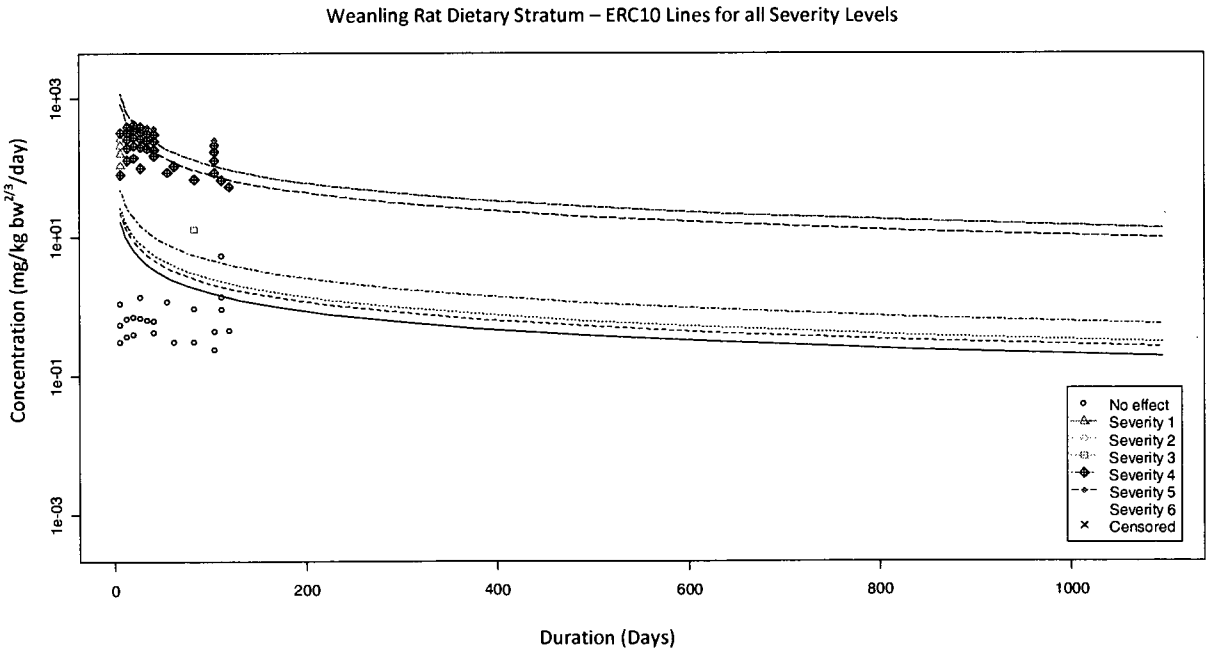

Figure G2: Cumulative odds model with the logit link function. Intercept stratified by the animal species and the route of copper exposure. Duration stratified by age.

Mature Rat Dietary Stratum

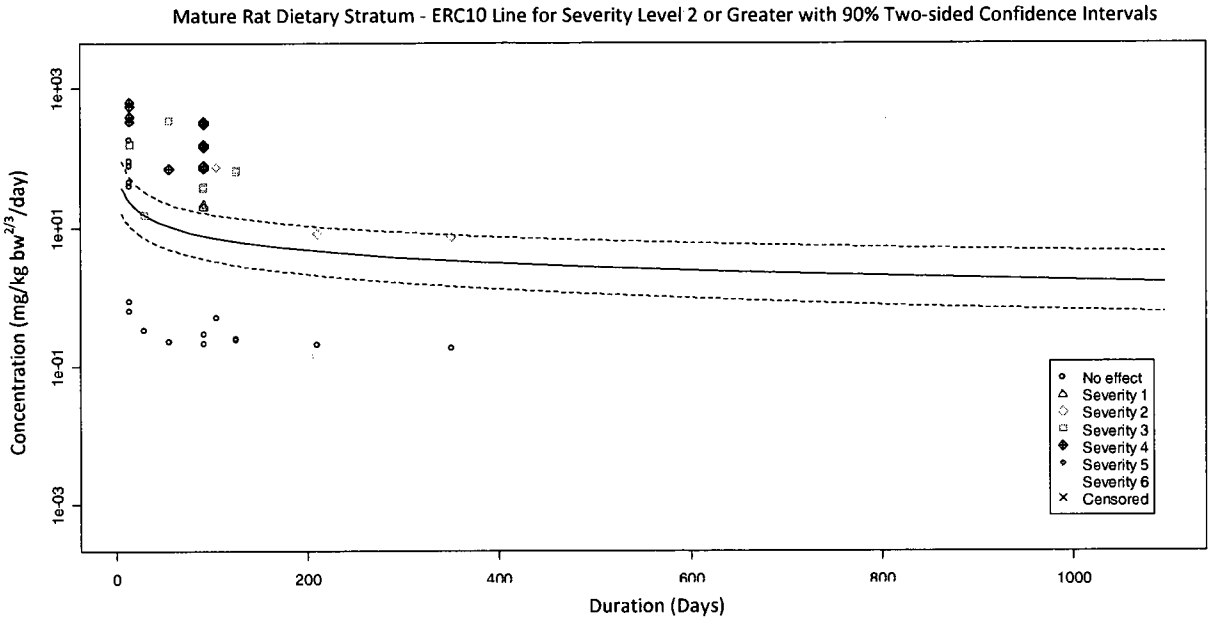

Figure G.3: Cumulative odds model with the logit link function. Intercept stratified by the animal species and the route of copper exposure. Duration stratified by age.

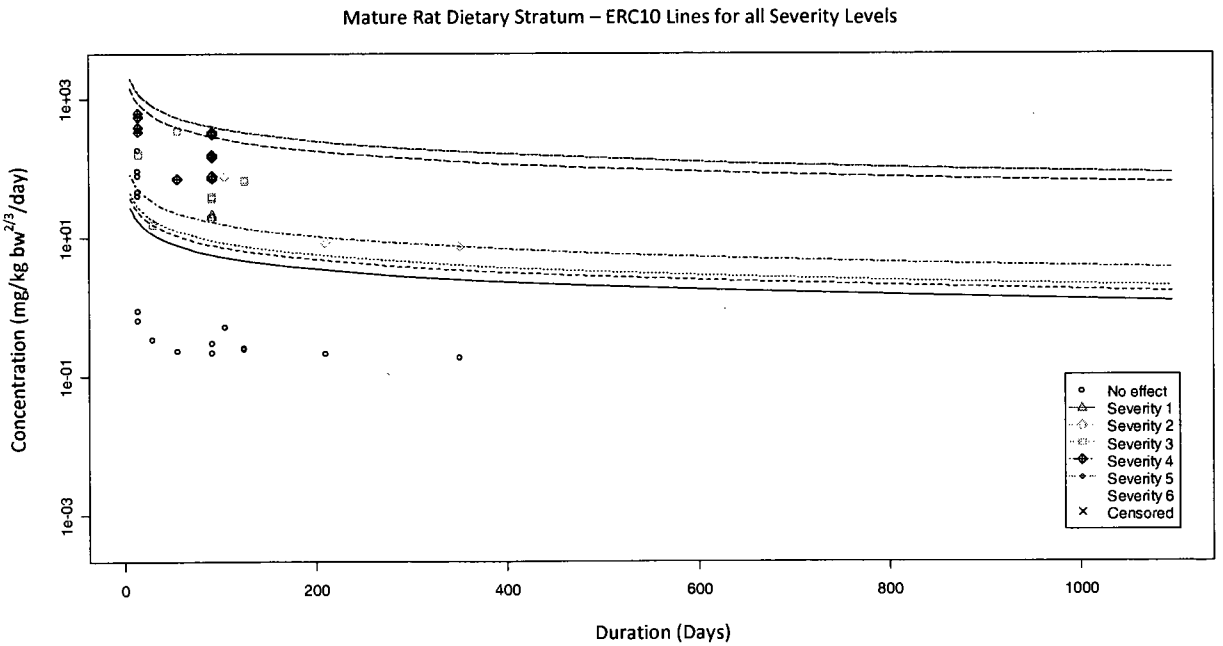

Figure G.4: Cumulative odds model with the logit link function. Intercept stratified by the animal species and the route of copper exposure. Duration stratified by age.

Mature Rat Drinking Water Stratum

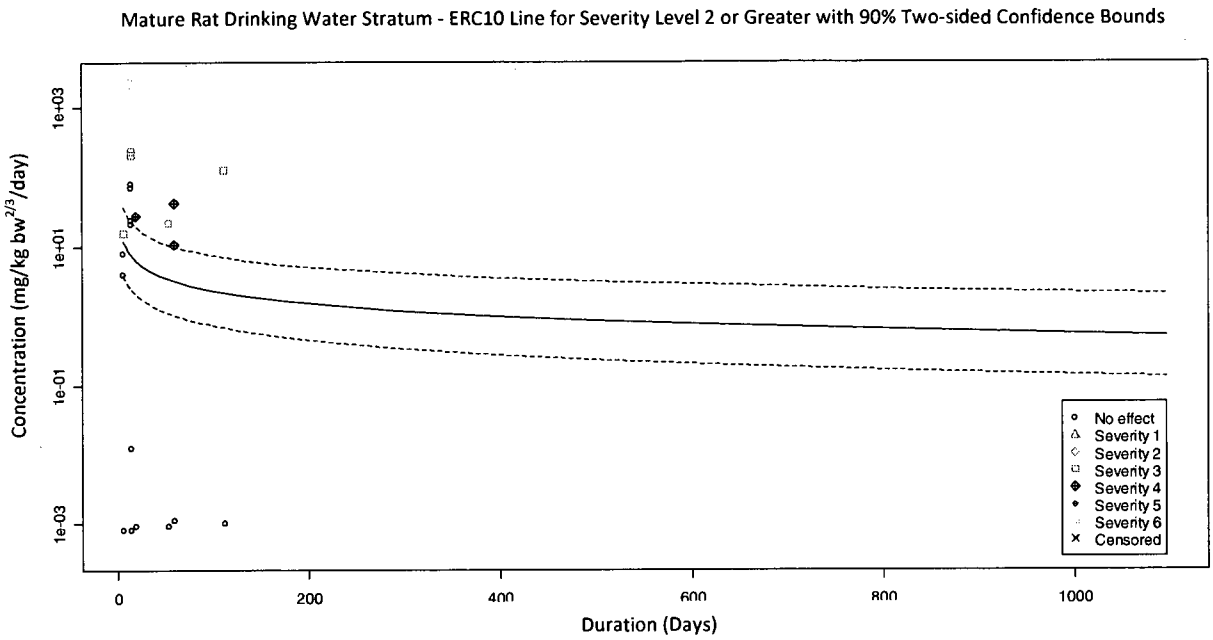

Figure G.5: Cumulative odds model with the logit link function. Intercept stratified by the animal species and the route of copper exposure. Duration stratified by age.

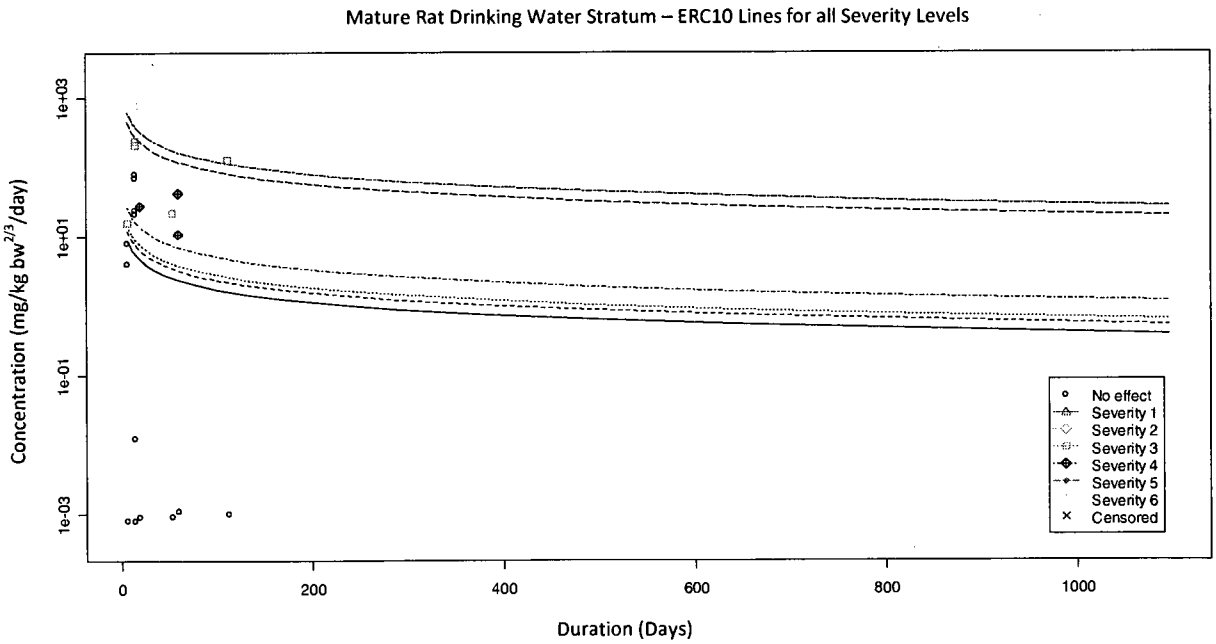

Figure G.6: Cumulative odds model with the logit link function. Intercept stratified by the animal species and the route of copper exposure. Duration stratified by age.

Weanling Mice Dietary Stratum

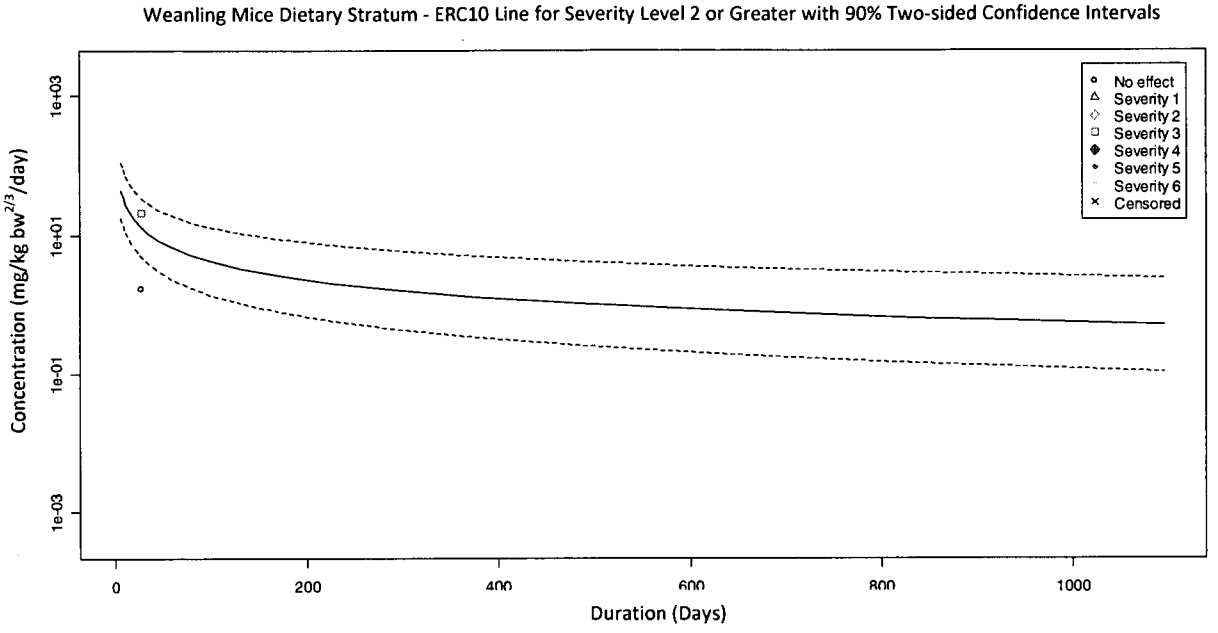

Figure G.7: Cumulative odds model with the logit link function. Intercept stratified by the animal species and the route of copper exposure. Duration stratified by age.

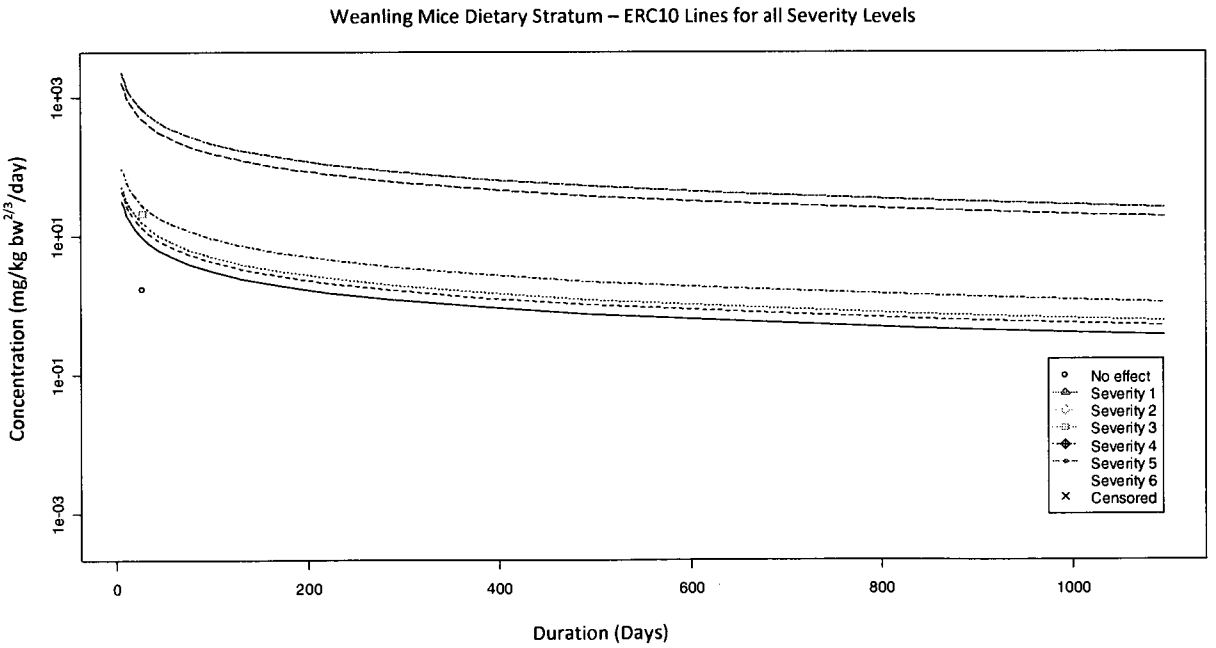

Figure G.8: Cumulative odds model with the logit link function. Intercept stratified by the animal species and the route of copper exposure. Duration stratified by age.

Adult Mice Dietary Stratum

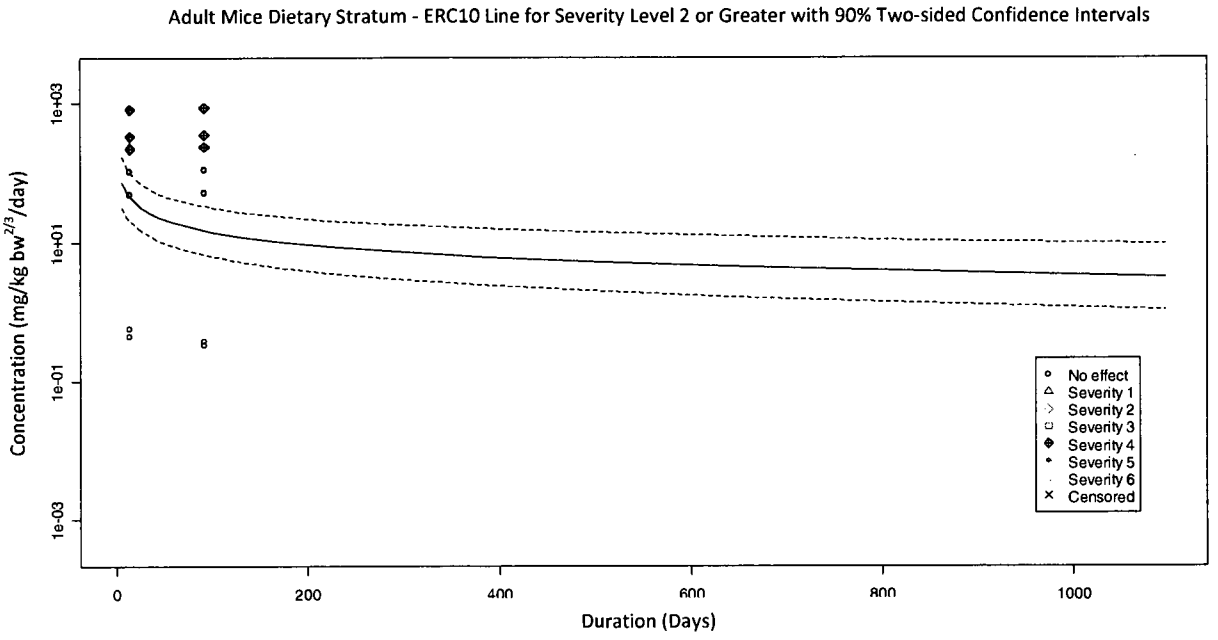

Figure G.9: Cumulative odds model with the logit link function. Intercept stratified by the animal species and the route of copper exposure. Duration stratified by age.

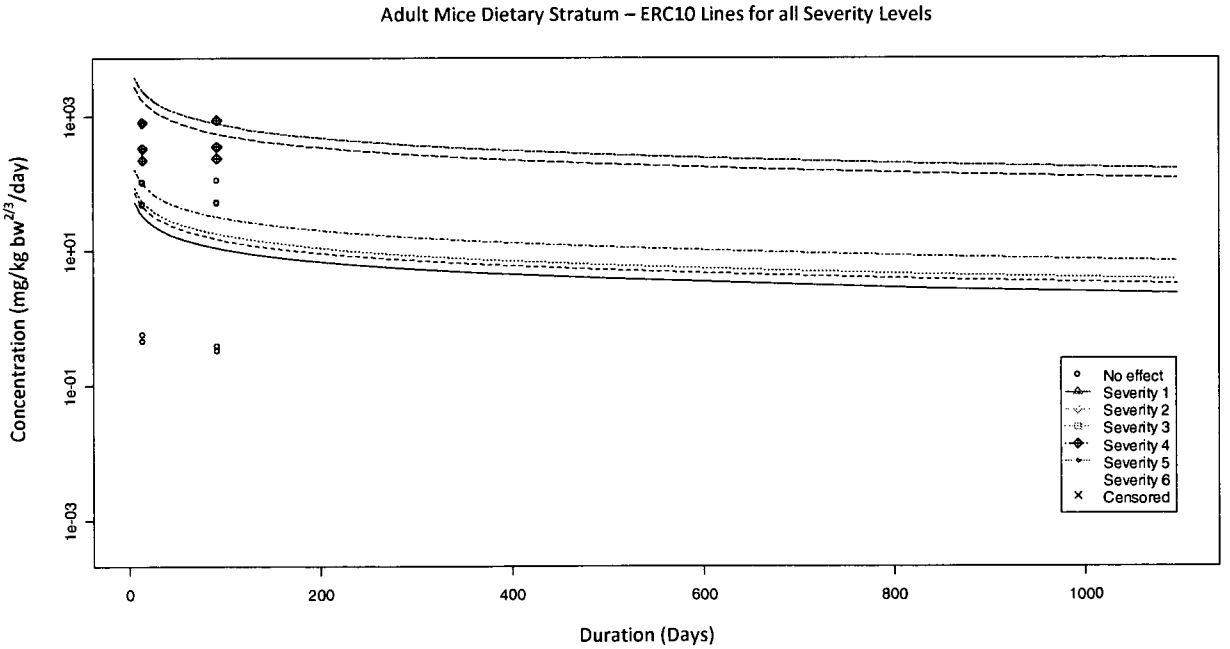

Figure G.10: Cumulative odds model with the logit link function. Intercept stratified by the animal species and the route of copper exposure. Duration stratified by age.

Mature Mice Drinking Water Stratum

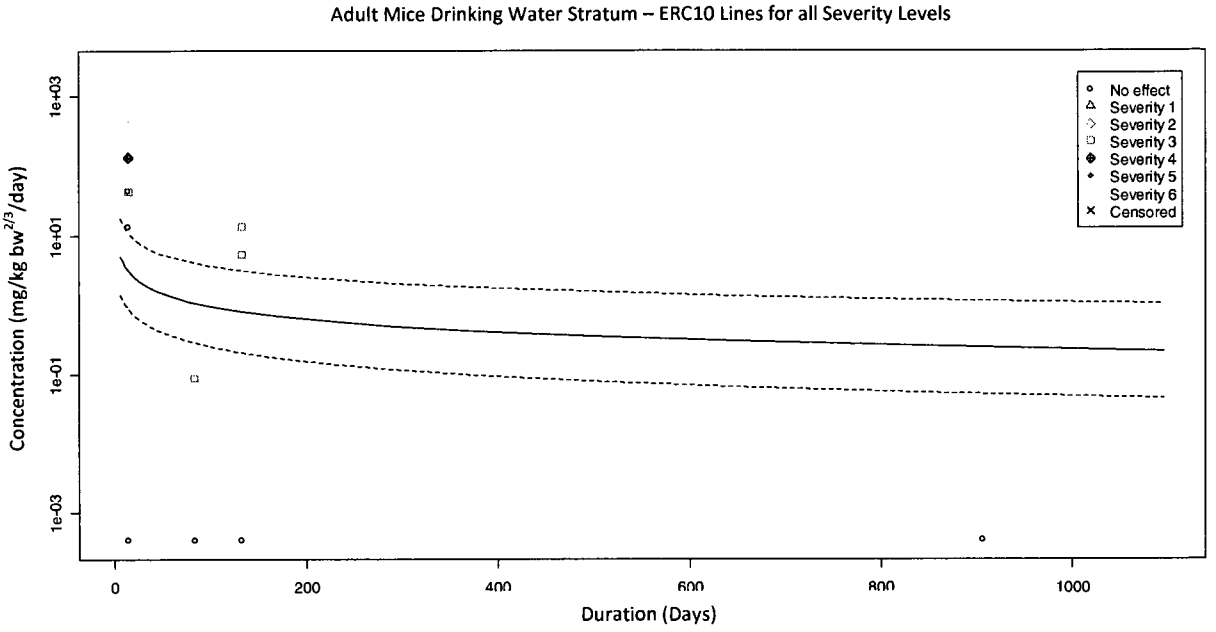

Figure G.11: Cumulative odds model with the logit link function. Intercept stratified by the animal species and the route of copper exposure. Duration stratified by age.

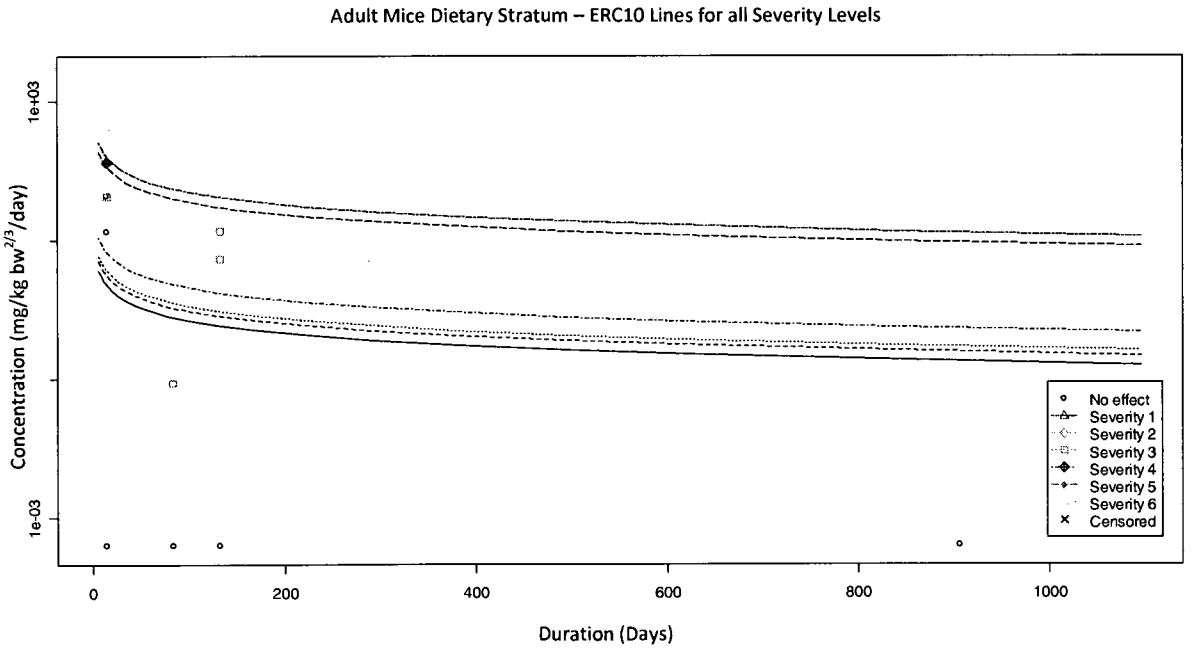

Figure G.12: Cumulative odds model with the logit link function. Intercept stratified by the animal species and the route of copper exposure. Duration stratified by age.

APPENDIX H: SEVERITY SCORE COMBINATIONS – UNRESTRICTED CUMULATIVE MODEL OF THE COPPER EXCESS DATA

Table H1 presents the original scoring scheme and a modified scoring scheme with six severity levels where severity level 0 and 1 have been combined. Table H2 presents the resulting parameter estimates, standard errors, Z-test statistics and p-values in the unrestricted cumulative model of the data with the modified scoring scheme. The resulting parameter estimates for the six severity levels are still incorrectly ordered (Table H2).

Table H1: Original and Modified Six-Level Severity Scoring Scheme

Original Scoring Scheme	*Modified Scoring Scheme*
0	0
1	
2	1
3	2
4	3
5	4
6	5

Table H2: Modified Six-Level Severity Scoring Scheme – Severity Coefficients, Estimates, Standard Errors, Z-test Statistics and P-values

Coefficients	*Estimates*	*Std. Error*	*Z-test=0*	*P-value*
SEV1	-4.9070	1.0489	-4.6785	<0.0001
SEV2	-2.3856	0.8798	-2.7116	0.0067
SEV3	-2.4808	0.8927	-2.7790	0.0055
SEV4	-12.9084	2.8533	-4.5240	<0.0001
SEV5	9.8390	11.6797	0.8424	0.3996

Cumulative odds model is used with the logit link function. Intercept stratified by the animal species.

Table H3 presents the original scoring scheme and a modified scoring scheme with five severity levels where severity level 0 and 1 have been combined. Table H4 presents the resulting parameter estimates, standard errors, Z-test statistics and p-values in the unrestricted cumulative model of the data with the modified scoring scheme. The resulting parameter estimates for the five severity levels are still incorrectly ordered (Table H4).

Table H3: Original and Modified Five-Level Severity Scoring Scheme

Original Scoring Scheme	*Modified Scoring Scheme*
0	0
1	
2	1
3	2
4	3
5	4
6	

Table H4: Modified Five-Level Severity Scoring Scheme – Severity Coefficients, Estimates, Standard Errors, Z-test Statistics and P-values

Coefficients	*Estimates*	*Std. Error*	*Z-test=0*	*P-value*
SEV1	-4.8846	1.0539	-4.6349	<0.0001
SEV2	-2.3071	0.8748	-2.6374	0.0084
SEV3	-2.2612	0.8969	-2.5211	0.0117
SEV4	-11.8679	3.2213	-3.6840	0.0002

Cumulative odds model uses the logit link function. Intercept stratified by the animal species.

Table H5 presents the original scoring scheme and a modified scoring scheme with four severity levels where severity level 0 and 1, 2 and 3 and 5 and 6 have been combined. Table H6 presents the resulting parameter estimates, standard errors, Z-test statistics and p-values in the unrestricted cumulative model of the data with the modified scoring scheme. The resulting parameter estimates for the five severity levels are still incorrectly ordered (Table H6)

Table H5: Original and Modified Four-Level Severity Scoring Scheme, Option 1

Original Scoring Scheme	*Modified Scoring Scheme*
0	0
1	
2	1
3	
4	2
5	3
6	

Table H6: Modified Four-Level Severity Scoring Scheme, Option 1 – Parameter Estimates, Standard Errors, Z-test Statistics and P-values

Coefficients	*Estimates*	*Std. Error*	*Z-test=0*	*P-value*
SEV1	-3.6525	0.8496	-4.2990	<0.0001
SEV2	-3.5222	0.7857	-4.4829	<0.0001
SEV3	-12.5122	3.2698	-3.8266	0.0001

Cumulative odds model uses the logit link function. Intercept stratified by the animal species.

Table H7 presents the original scoring scheme and a second option for a modified scoring scheme with four severity levels where severity level 0 and 1, 3 and 4 and 5 and 6 have been combined. Table H8 presents the resulting parameter estimates, standard errors, Z-test statistics and p-values in the unrestricted cumulative model of the data with the modified scoring scheme. The resulting parameter estimates for the five severity levels are still incorrectly ordered.

Table H7: Original and Modified Four-Level Severity Scoring Scheme, Option 2

Original Scoring Scheme	*Modified Scoring Scheme*
0	0
1	
2	1
3	2
4	
5	3
6	

Table H8: Modified Three-Level Severity Scoring Scheme, Option 2 – Severity Coefficients, Estimates, Standard Errors, Z-test Statistics and P-values

Coefficients	*Estimates*	*Std. Error*	*Z-test=0*	*P-value*
SEV1	-4.8015	0.9744	-4.9275	<0.0001
SEV2	-2.5783	0.8929	-2.8876	0.0039
SEV3	-12.2710	3.1878	-3.8494	0.0002

Cumulative odds model uses the logit link function. Intercept stratified by the animal specie.

Table H9 presents the original scoring scheme and a modified scoring scheme with three severity levels where severity level 0 and 1, 2-4 and 5 and 6 have been combined. Table H10 presents the resulting parameter estimates, standard errors, Z-test statistics and p-values in the unrestricted cumulative model of the data with the modified scoring scheme. The resulting parameter estimates for the three severity levels are still incorrectly ordered.

Table H9: Original and Modified Three-Level Severity Scoring Scheme, Option 1

Original Scoring Scheme	*Modified Scoring Scheme*
0	0
1	
2	1
3	
4	
5	2
6	

Table H10: Modified Three-Level Severity Scoring Scheme, Option 1 – Severity Coefficients, Estimates, Standard Errors, Z-test Statistics and P-values

Coefficients	*Estimates*	*Std. Error*	*Z-test=0*	*P-value*
SEV1	-5.3079	1.1356	-4.4747	<0.0001
SEV2	-12.9378	3.2586	-3.9704	<0.0001

Cumulative odds model uses the logit link function. Intercept stratified by the animal species.

Table H11 presents the original scoring scheme and second option for a modified scoring scheme with three severity levels where severity level 0 and 1, 2 and 3 and 4-6 have been combined. Table H12 presents the resulting parameter estimates, standard errors, Z-test statistics and p-values in the unrestricted cumulative model of the data with the modified scoring scheme. The resulting parameter estimates for the three severity levels are still incorrectly ordered (H12).

Table H11: Original and Modified Three-Level Severity Scoring Scheme, Option 2

Original Scoring Scheme	*Modified Scoring Scheme*
0	0
1	
2	1
3	
4	2
5	
6	

Table H12: Modified Three-Level Severity Scoring Scheme, Option 2 – Severity Coefficients, Estimates, Standard Errors, Z-test Statistics and P-values

Coefficients	*Estimates*	*Std. Error*	*Z-test=0*	*P-value*
SEV1	-3.6780	0.8563	-4.2954	<0.0001
SEV2	-3.5588	0.7982	-4.4587	<0.0001

Cumulative odds model uses the logit link function. Intercept stratified by the animal species.

APPENDIX I: ERC10 PLOTS FOR RATS AND MICE WITH THE TWO-LEVEL SEVERITY MODEL FOR COPPER EXCESS

Mature Rat Dietary Stratum

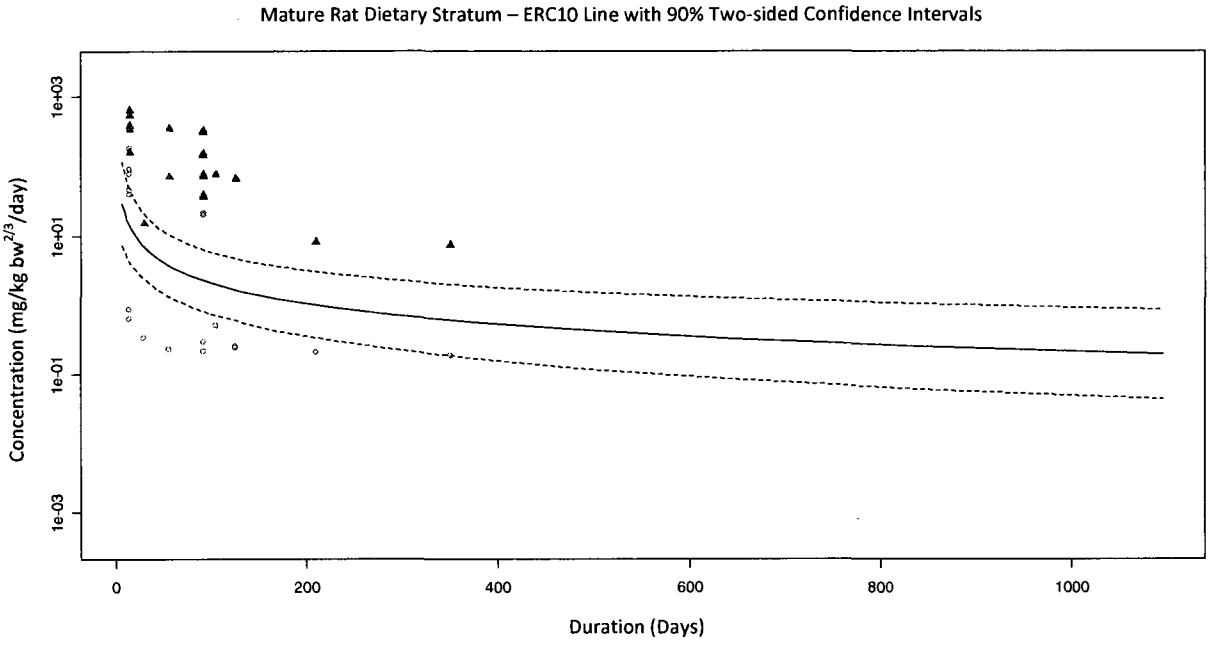

Figure I1: ◌ = severity level 0, ▲ = severity level 1. Two level severity model with the logit link function. Concentration and duration log transformed. The concentration parameter is stratified by animal species, route of exposure and age and the duration parameter is stratified by the animal species and route of exposure.

Weanling Rat Dietary Stratum

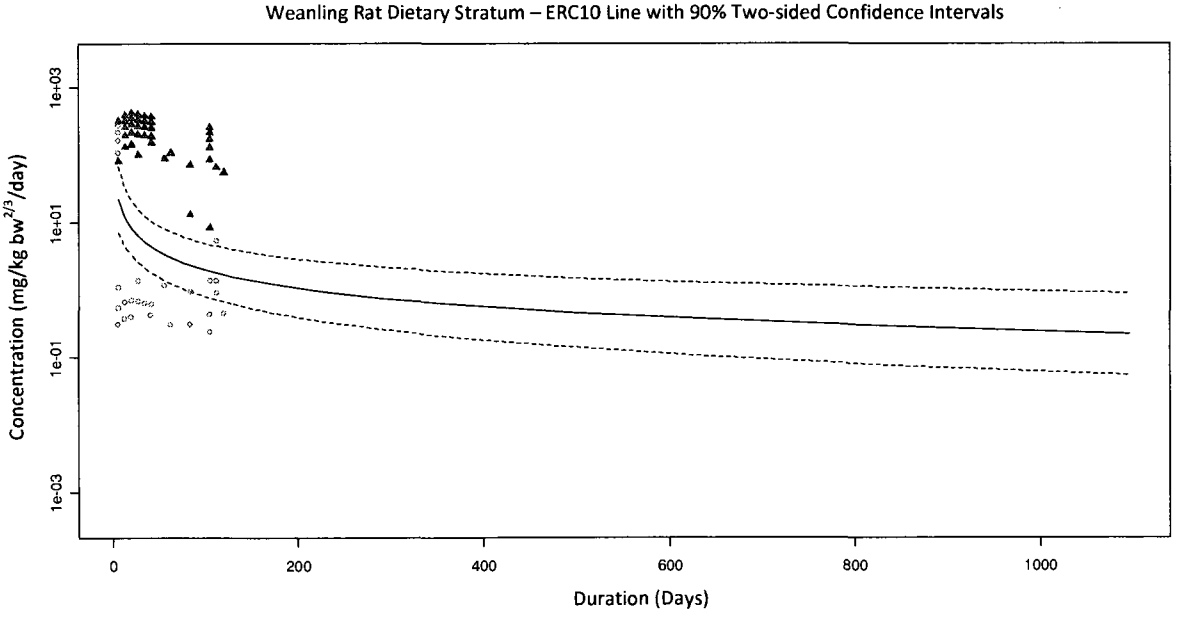

Figure I2: ◌ = severity level 0, ▲ = severity level 1. Two level severity model with the logit link function. Concentration and duration log transformed.

Mature Rat Drinking Water Stratum

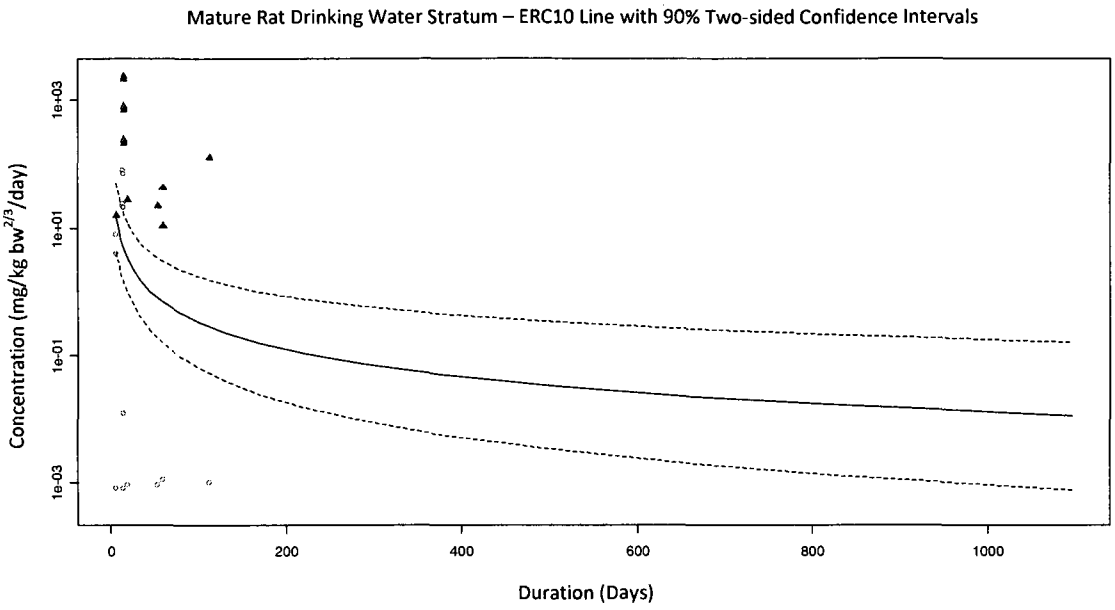

Figure I3: ◌ = severity level 0, ▲ = severity level 1. Two level severity model with the logit link function. Concentration and duration log transformed. The concentration parameter is stratified by animal species, route of exposure and age and the duration parameter is stratified by the animal species and route of exposure.

Mature Mice Dietary Studies

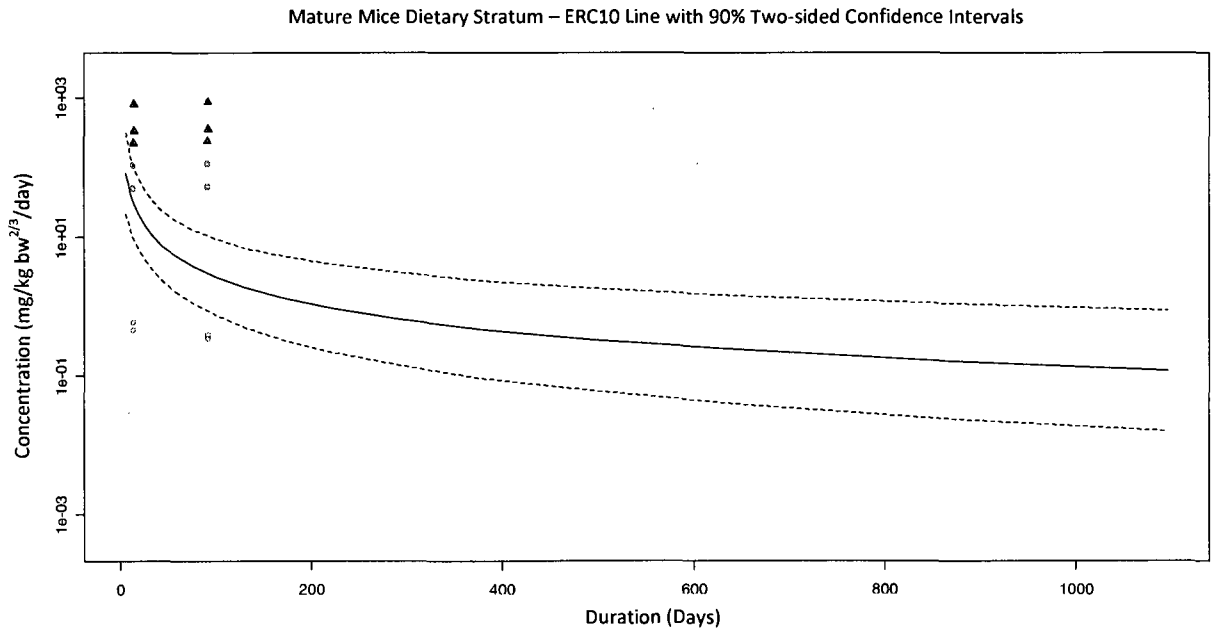

Figure I4: ◌ = severity level 0, ▲ = severity level 1. Two level severity model with the logit link function. Concentration and duration log transformed. The concentration parameter is stratified by animal species, route of exposure and age and the duration parameter is stratified by the animal species and route of exposure.

Weanling Mice Dietary Studies

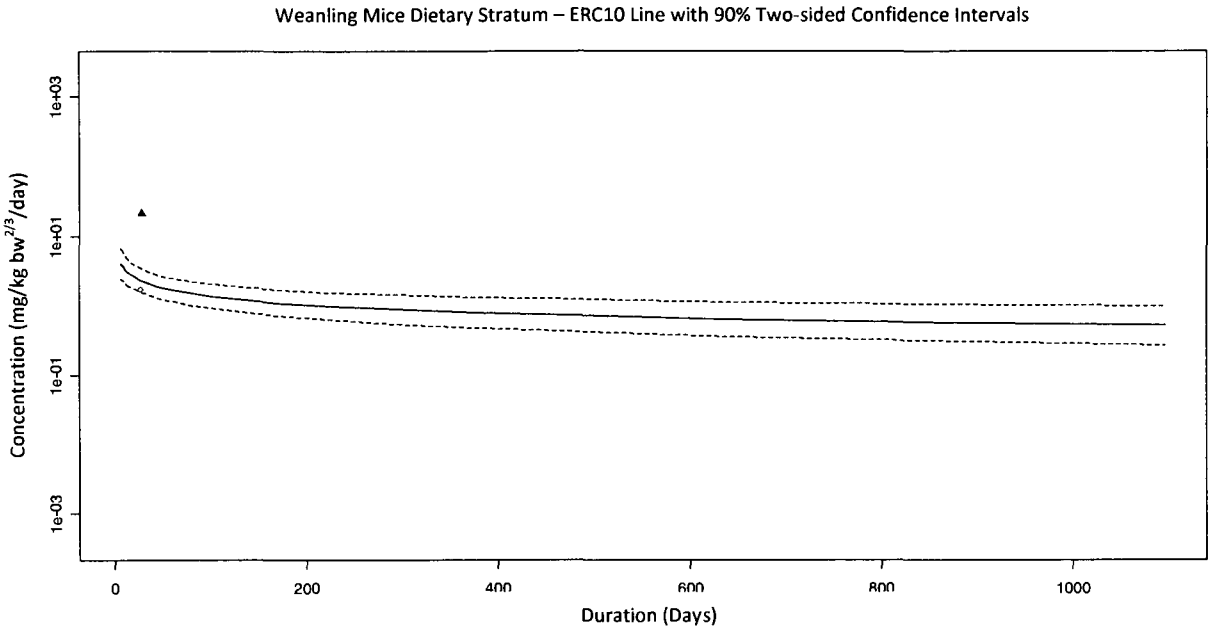

Figure I5: ◌ = severity level 0, ▲ = severity level 1. Two level severity model with the logit link function. Concentration and duration log transformed. The concentration parameter is stratified by animal species, route of exposure and age and the duration parameter is stratified by the animal species and route of exposure.

Mature Mice Drinking Water Studies

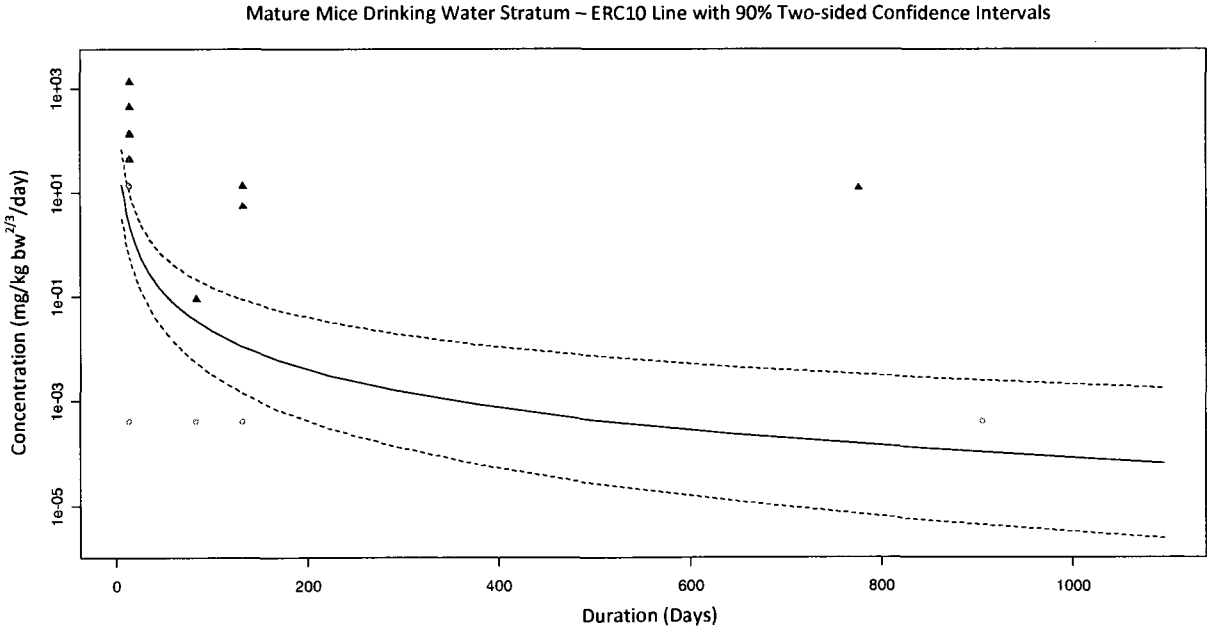

Figure I6: ◌ = severity level 0, ▲ = severity level 1. Two level severity model with the logit link function. Concentration and duration log transformed. The concentration parameter is stratified by animal species, route of exposure and age and the duration parameter is stratified by the animal species and route of exposure.

APPENDIX J: ERC10 PLOTS FOR RATS AND MICE WITH THE CUMULATIVE ODDS MODEL FOR COPPER DEFICIENCY

Mature Rat Stratum

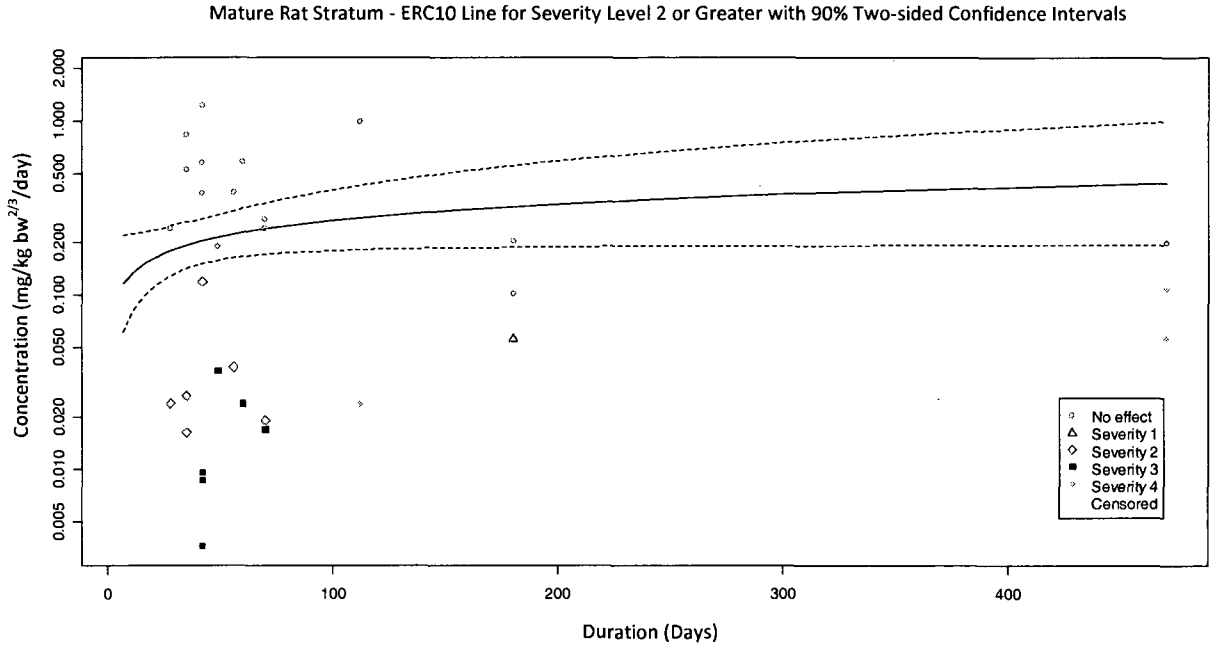

Figure 4.J1: ERC10 line for severity level 2 or greater with 90% two-sided confidence intervals for the mature rat stratum. Cumulative odds model with the probit link function transforms concentration (mg/kg $bw^{2/3}$/day) and duration (days) to the base 10. Intercept stratified by animal species and concentration by age.

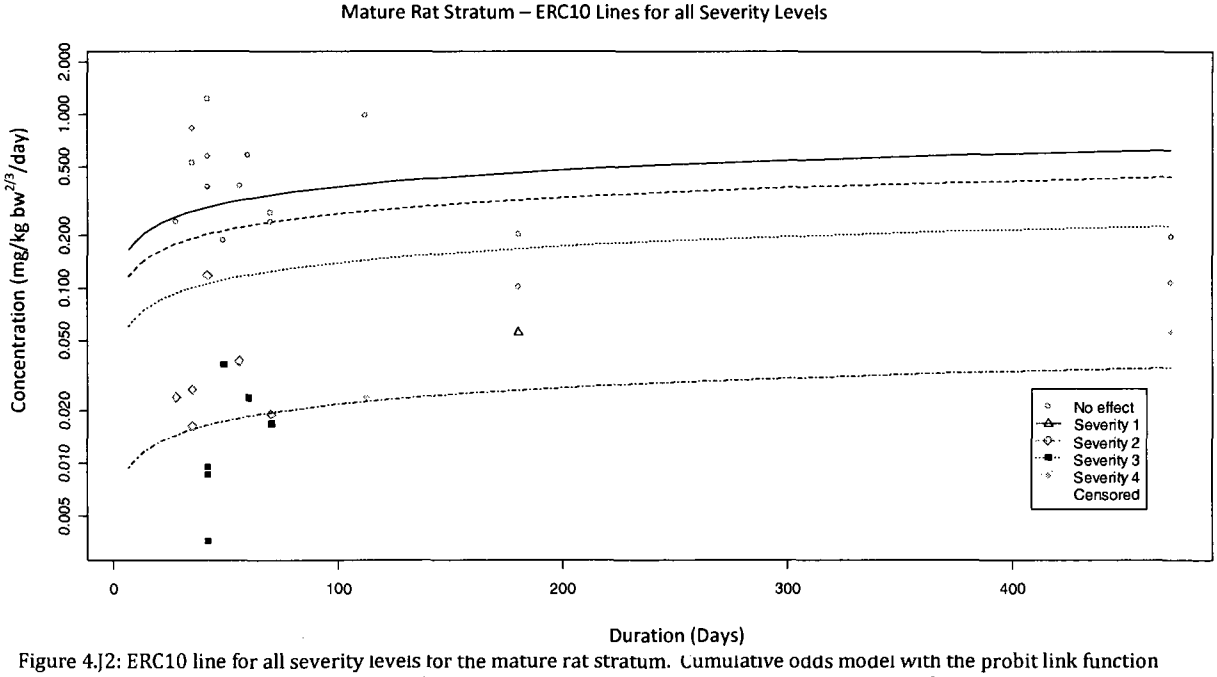

Figure 4.J2: ERC10 line for all severity levels for the mature rat stratum. Cumulative odds model with the probit link function transforms concentration (mg/kg $bw^{2/3}$/day) and duration (days) to the base 10. Intercept stratified by animal species and concentration by age.

Weanling Rat Stratum

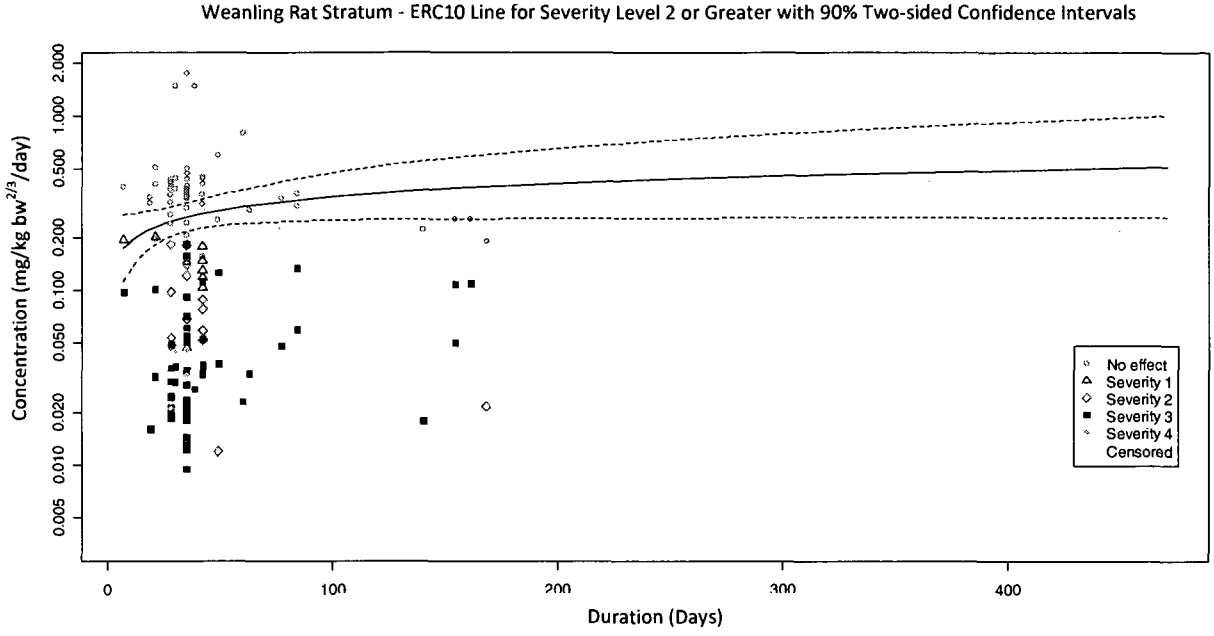

Figure 4.J3: ERC10 line for severity level 2 or greater with 90% two-sided confidence intervals for the weanling rat stratum. Cumulative odds model with the probit link function transforms concentration (mg/kg bw$^{2/3}$/day) and duration (days) to the base 10. Intercept stratified by animal species and concentration by age.

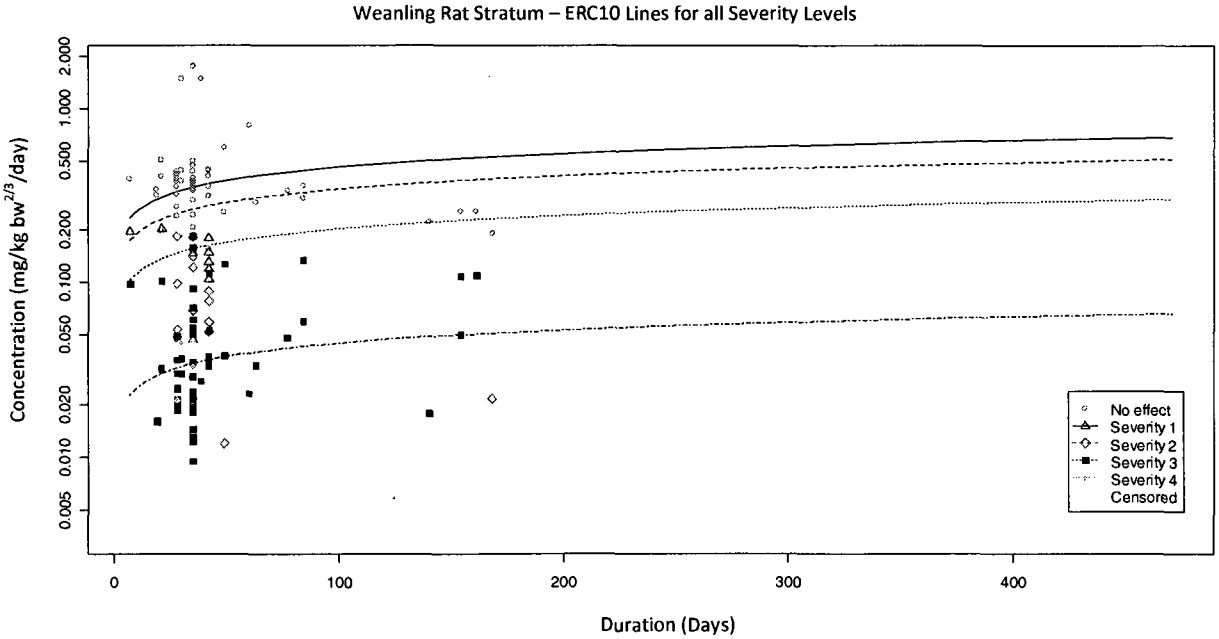

Figure 4.J4: ERC10 line for all severity levels for the weanling rat stratum. Cumulative odds model with the probit link function transforms concentration (mg/kg bw$^{2/3}$/day) and duration (days) to the base 10. Intercept stratified by animal species and concentration by age.

Mature Mice Stratum

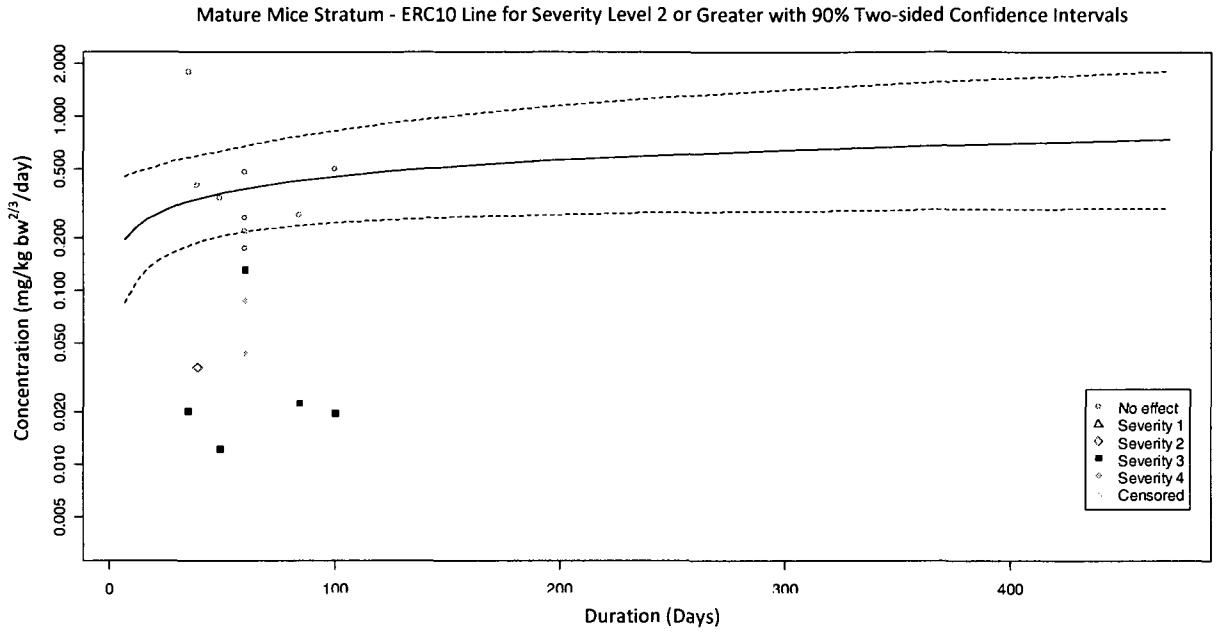

Figure 4.J5: ERC10 line for severity level 2 or greater with 90% two-sided confidence intervals for the mature mice stratum. Cumulative odds model with the probit link function transforms concentration (mg/kg $bw^{2/3}$/day) and duration (days) to the base 10. Intercept stratified by animal species and concentration by age.

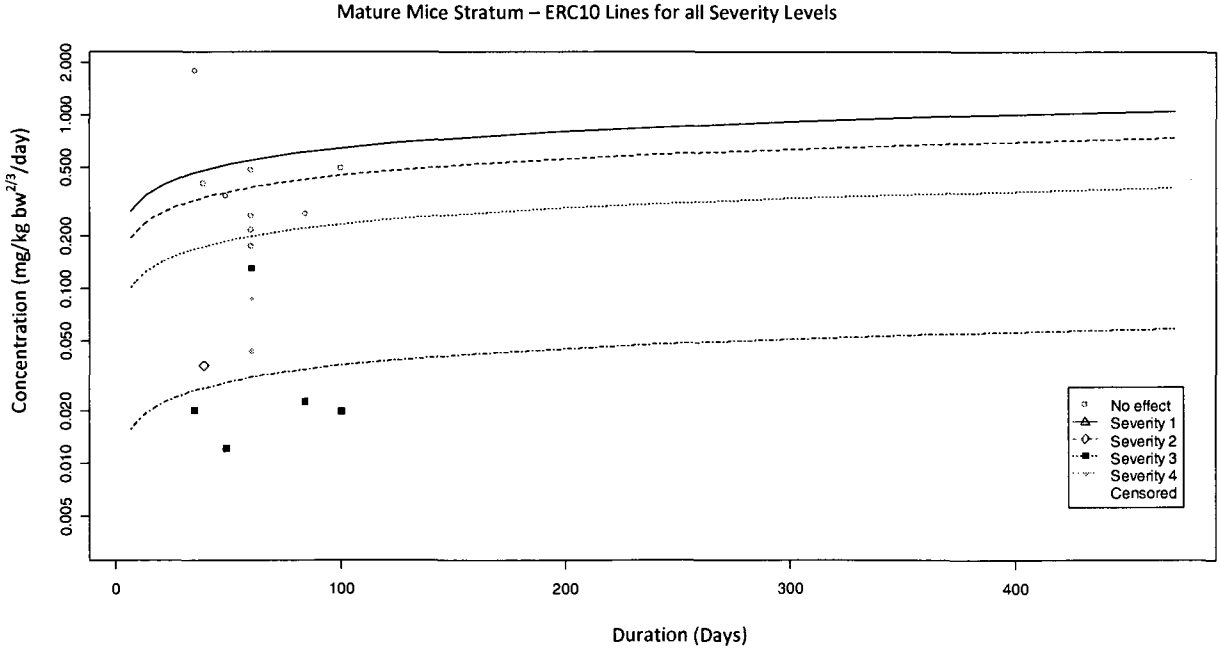

Figure 4.J6: ERC10 line for all severity levels for the mature mice stratum. Cumulative odds model with the probit link function transforms concentration (mg/kg $bw^{2/3}$/day) and duration (days) to the base 10. Intercept stratified by animal species and concentration by age.

Weanling Mice Stratum

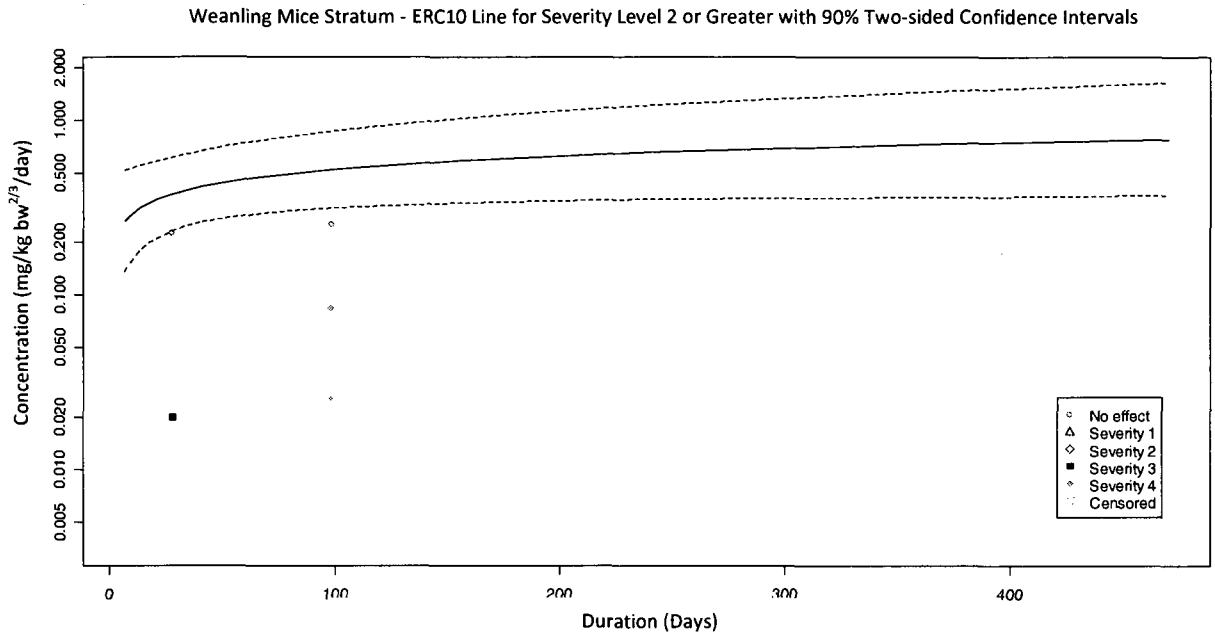

Figure 4.J7: ERC10 line for severity level 2 or greater with 90% two-sided confidence intervals for the weanling mice stratum. Cumulative odds model with the probit link function transforms concentration (mg/kg bw$^{2/3}$/day) and duration (days) to the base 10. Intercept stratified by animal species and concentration by age.

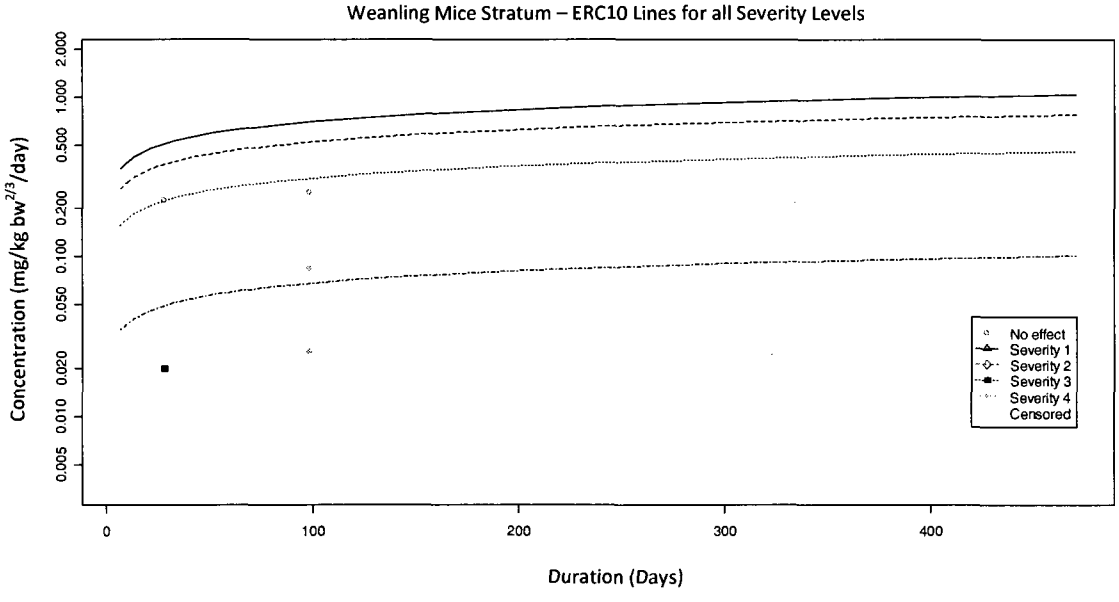

Figure 4.J8: ERC10 line for all severity levels for the weanling mice stratum. Cumulative odds model with the probit link function transforms concentration (mg/kg bw$^{2/3}$/day) and duration (days) to the base 10. Intercept stratified by animal species and concentration by age.

APPENDIX K: SEVERITY SCORE COMBINATIONS – UNRESTRICTED CUMULATIVE MODEL OF THE COPPER DEFICIENCY DATA

Table K1 presents the original scoring scheme and a modified scoring scheme with four severity levels where severity level 3 and 4 have been combined. Table K2 presents the resulting parameter estimates, standard errors, Z-test statistics and p-values in the unrestricted cumulative model of the data with the modified scoring scheme. The resulting parameter estimates for the four severity levels are still incorrectly ordered (K2).

Table K1: Original and Modified Four-Level Severity Scoring Scheme, Option 1

Original Scoring Scheme	*Modified Scoring Scheme*
0	0
1	1
2	2
3	3
4	

Table K2: Modified Four-Level Severity Scoring Scheme, Option 1 – Severity Coefficients, Estimates, Standard Errors, Z-test Statistics and P-values

Coefficients	*Estimates*	*Std. Error*	*Z-test=0*	*P-value*
SEV1	-7.9411	2.1908	-3.6247	0.0003
SEV2	-3.0920	1.7343	-1.7829	0.07461
SEV3	-4.8422	1.3624	-3.5541	0.00038

Unrestricted cumulative model uses the probit link function. Intercept stratified by the animal species.

Table K3 presents the original scoring scheme and a second option for a modified scoring scheme with four severity levels where severity level 0 and 1 have been combined. Table K4 presents the resulting parameter estimates, standard errors, Z-test statistics and p-values in the unrestricted cumulative model of the data with the modified scoring scheme. The resulting parameter estimates for the four severity levels are still incorrectly ordered.

Table K3: Original and Modified Four-Level Severity Scoring Scheme, Option 2

Original Scoring Scheme	*Modified Scoring Scheme*
0	0
1	
2	1
3	2
4	3

Table K4: Modified Four-Level Severity Scoring Scheme, Option 2 – Severity Coefficients, Estimates, Standard Errors, Z-test Statistics and P-values

Coefficients	*Estimates*	*Std. Error*	*Z-test=0*	*P-value*
SEV1	-6.9895	1.0152	-6.8851	<0.0001
SEV2	-4.0030	0.6382	-6.2722	<0.0001
SEV3	-5.5606	0.5566	-9.9904	<0.0001

Unrestricted cumulative model uses the probit link function. Intercept stratified by the animal species.

Table K5 presents the original scoring scheme and a third option for a modified scoring scheme with four severity levels where severity level 2 and 3 have been combined. Table K6 presents the resulting parameter estimates, standard errors, Z-test statistics and p-values in the unrestricted cumulative model of the data with the modified scoring scheme. The resulting parameter estimates for the four severity levels are still incorrectly ordered (Table K6).

Table K5: Original and Modified Four-Level Severity Scoring Scheme, Option 3

Original Scoring Scheme	*Modified Scoring Scheme*
0	0
1	1
2	2
3	
4	3

Table K6: Modified Four-Level Severity Scoring Scheme, Option 3 – Severity Coefficients, Estimates, Standard Errors, Z-test Statistics and P-values

Coefficients	*Estimates*	*Std. Error*	*Z-test=0*	*P-value*
SEV1	-10.2948	2.3636	-4.3555	<0.0001
SEV2	-6.2880	1.2862	-4.8886	<0.0001
SEV3	-5.3199	0.5345	-9.9525	<0.0001

Unrestricted cumulative model uses the probit link function. Intercept stratified by the animal species.

Table K7 presents the original scoring scheme and a modified scoring scheme with three severity levels where severity level 0 and 1 and severity level 3 and 4 have been combined. Table K8 presents the resulting parameter estimates, standard errors, Z-test statistics and p-values in the unrestricted cumulative model of the data with the modified scoring scheme. The resulting parameter estimates for the three severity levels are still incorrectly ordered (Table K8).

Table K7: Original and Modified Three-Level Severity Scoring Scheme, Option 1

Original Scoring Scheme	*Modified Scoring Scheme*
0	0
1	
2	1
3	2
4	

Table K8: Modified 3-Level Severity Scoring Scheme, Option 1 – Severity Coefficients, Estimates, Standard Errors, Z-test Statistics and P-values

Coefficients	*Estimates*	*Std. Error*	*Z-test=0*	*P-value*
SEV1	-7.0463	1.0121	-6.9625	<0.0001
SEV2	-4.2198	0.6705	-6.2933	<0.0001

Unrestricted cumulative model uses the probit link function. Intercept stratified by the animal species.

Table K9 presents the original scoring scheme and a second option for a modified scoring scheme with three severity levels where severity level 0 and 1 and severity level 2 and 3 have been combined. Table K10 presents the resulting parameter estimates, standard errors, Z-test statistics and p-values in the unrestricted cumulative model of the data with the modified scoring scheme. The resulting parameter estimates for the three severity levels are still incorrectly ordered (Table K10).

Table K9: Original and Modified Three-Level Severity Scoring Scheme, Option 2

Original Scoring Scheme	*Modified Scoring Scheme*
0	0
1	
2	1
3	
4	2

Table K10: Modified Three-Level Severity Scoring Scheme, Option 2 – Severity Coefficients, Estimates, Standard Errors, Z-test Statistics and P-values

Coefficients	*Estimates*	*Std. Error*	*Z-test=0*	*P-value*
SEV1	-7.4962	1.3111	-5.7175	<0.0001
SEV2	-5.9885	0.4561	-13.1302	<0.0001

Unrestricted cumulative model uses the probit link function. Intercept stratified by the animal species.

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---

* Web site address

* www.mclaughlincentre.ca

---

* Type of organization/affiliation

* Academic

---

* If other, please specify

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---

* Position

* Student

---

* Telephone

* +613-592-3745

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