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**PROCEEDINGS OF THE 1996
CONFERENCE ON ADVANCES IN
TOXICOLOGY AND APPLICATIONS
TO RISK ASSESSMENT**

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This technical report has been reviewed and is approved for publication.

FOR THE DIRECTOR



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PREFACE

This special report presents select papers and abstracts from the 1996 Conference on Advances in Toxicology and Applications to Risk Assessment held at the Hope Hotel and Conference Center at Wright-Patterson Air Force Base, OH, from 23-25 April 1996. The conference was coordinated by ManTech Environmental Technology, Inc., Toxic Hazards Research, under Department of the Air Force Contract No. F41625-96-C-9010, a joint venture contract operated by ManTech Environmental Technology, Inc. and Geo-Centers, Inc. Lt Col Terry Childress served as the Contracting Officer's Representative for the U.S. Air Force, Armstrong Laboratory. Darol E. Dodd, Ph.D., served as Program Manager for the Toxic Hazards Research.

Appreciation and thanks are extended to the authors who contributed papers to this special report, the colleagues who reviewed the papers, and the personnel from Toxic Hazards Research, Air Force, Army, Navy, EPA, and ATSDR who participated in the preparation and coordination of the conference. Special acknowledgment is extended to Ms. Lois Doncaster, Conference Coordinator, and her support staff for outstanding dedication and devotion toward making the conference a highly successful event.

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INTRODUCTION

David R. Mattie
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The Toxicology Division of the Occupational and Environmental Health Directorate of the Armstrong Laboratory sponsored its annual conference related to toxicology and risk assessment issues and science on 23 to 25 April 1996. The "Conference on Advances in Toxicology and Applications to Risk Assessment," was held at the Hope Hotel and Conference Center located on Wright-Patterson AFB. This interagency conference was also sponsored by the Navy Medical Research Institute Detachment (Toxicology); the Army Medical Research Detachment, Wright-Patterson AFB; the Division of Toxicology, Agency for Toxic Substances and Disease Registry; and the United States Environmental Protection Agency (EPA/NCEA Cincinnati, OH and Washington DC).

The goals of the conference were: (1) exploration of new methodologies for human and ecological risk assessments; (2) application of guidelines and models in the risk assessment process; and (3) examination of the issues and approaches for communication risk. The conference was divided into five sessions: (1) Environmental Risk Assessment Program (ERAP); (2) Ecological Risk: Assessment, Incorporation, and Application; (3) Biologically Based Modeling Applications in Risk Assessment of Toxic Substances; (4) Risk-based Guidelines (Applications of Reference Values); and (5) Risk Communication in the Federal Government. The conference also featured a poster session for studies relevant to the themes as well as a database/modeling demonstration preview.

The papers presented in this special report, though not inclusive of the entire conference, are representative of the goals and sessions of the conference. The abstracts of all oral and poster presentations are included in order to present a complete overview of the entire conference. The title of each session is a good description of the type of presentations made in that session.

The first session was devoted to the ERAP or Environmental Risk Assessment Program. The ERAP was a joint Department of Defense (DOD), Department of Energy (DOE), and Environmental Protection Agency (EPA) interagency group to advise the EPA on risk assessment issues related to hazardous waste sites. The intent of the group is to improve the scientific process for evaluating the nature and degree of threat posed by specific contaminants so that decisions include the best available evaluation of human health and ecological risk. This protects not only public health and the environment but was intended to address sites specific to the military and DOE. The paper by Gunda Reddy is a success story for the ERAP.

The second session examined a relatively new area within the military, ecotoxicology. The presentations looked at ecological risk from both a DOD and EPA perspective. The papers by Terry L. Walker and Mark S. Johnson, et al, included in this report, are only from the Army but are still every representative of the issues in ecotoxicology. In the third session the four presentations examined the use of biologically based models for risk assessment. The papers on noncancer risk assessment by Harvey Clewell III and pregnancy by John F. Young, et al, are excellent examples of how biologically based models can impact the risk assessment process. The fourth session discussed risk-based guidelines or reference values/standards. Although only one paper by Chris DeRosa and John Risher was submitted for this report, it presents a number of reference values, how they are derived and intended to be used in the risk assessment process. Risk communication becomes more critical every year. The topic of the fifth session, risk communication, was actually a topic at the 1994 conference. Once again it was a popular and informative session. This report contains papers from four of the six presentations, the most representative collections of papers for all of the sessions. Papers were written by Steven E. Williams, Tim L. Tinker, John T. Paul, Jr., and Marie T. Flickinger.

Once again, the conference was a success due to the dedication and work of Lois Doncaster and her support staff at ManTech Environmental Technology, Inc. The editors would like to thank Lois and her staff for all their efforts. The editors would like to thank Sheila Brooks and Susie Godfrey for their hard work and invaluable assistance in beginning the process to compile this report. The editors would also like to thank Teresa Ellis for her work and assistance in completing the process of publishing this report.

SESSION 1
ENVIRONMENTAL RISK ASSESSMENT PROGRAM
(ERAP)

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**ASSESSMENT OF ENVIRONMENTAL HAZARDS OF
1,3,5-TRINITROBENZENE (TNB)**

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ABSTRACT

The remedial investigation/feasibility studies conducted at certain Army installations showed a need to clean up contaminated sites, where high levels of ammunition chemicals such as 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB), 1,3-dinitrobenzene (DNB) and their degradation products/metabolites were detected in surface soil and groundwater. TNB is a photo degradation product of TNT; it is not easily degraded, and persists in the environment. The toxicity data on TNB are scanty. Hence US Environmental Protection Agency (USEPA) (1988) developed a reference dose (RfD) for TNB (0.00005 mg/kg/day for chronic toxicity) based on the toxicity of DNB, which is structurally similar to TNB. Since then we have completed acute, subacute, subchronic, chronic, reproductive and developmental toxicity studies and toxicokinetics studies. We have reviewed the mammalian toxicity data for TNB and have determined the No Observed Adverse Effect Levels (NOAEL) and Low Observed Adverse Effect Levels (LOAEL), for subchronic, chronic, reproductive and developmental toxicity. Based on the newly determined NOAEL and LOAEL values, we have now developed a new RfD for TNB (0.03 mg/kg/day), based on the chronic toxic effects on hematology and histopathological changes in testes and kidney.

INTRODUCTION

1,3,5-trinitrobenzene (TNB), a nitroaromatic compound, is prepared by the decarboxylation of trinitrobenzoic acid or by the oxidation of 2,4,6-trinitrotoluene. TNB is a Class A explosive that is less sensitive to impact but more powerful and brisant than 2,4,6-trinitrotoluene (TNT) (Budavari et al., 1989; Fedoroff et al., 1962). TNB is used primarily in explosive compositions and munitions and has had limited use in the vulcanization of rubber (Barnhart, 1981). However most TNB contamination results from photo degradation of TNT. TNB has been detected as an environmental contaminant of surface water, ground water and soil near production waste disposal sites, and certain Army installations. The waste waters discharged from TNT manufacturing processes contain a large number of nitro aromatic compounds, including TNB. TNB is formed during the nitration step of TNT synthesis as a result of oxidation of methyl groups. Although the complete mechanism of TNB formation during TNT photolysis in the environment is unknown, it has been suggested that it is produced from decarboxylation of 2,4,6-trinitrobenzaldehyde, a major TNT photo product (Burlinson, 1980). TNB is also found in aquatic systems as a by-product of biotransformation and photolysis of TNT. TNB is not readily biodegradable; it persists in the environment and has a high propensity to leach out and contaminate groundwater near production waste disposal sites, and in soils at certain U.S. Army installations (Walsh and Jenkins, 1992) and hazardous waste sites (Garman et al., 1987).

Problem definition studies revealed that the toxicity data are limited on many of the nitroaromatic compounds related to production of munitions. The toxicity data on TNB are limited to a few

abstracts describing oral LD50 values published in Russian (Timofievskaya and Rodinova 1973; Korolev et al., 1977). Since there was no toxicity data on TNB, the RfD for TNB was derived based on the toxicity of 1,3-dinitrobenzene which is structurally similar to TNB (U.S. EPA 1988). These values were used by the Army to clean up/remediate sites contaminated with TNB. The cost for this clean up and remediation were found to be high. The estimated cost for 1 cubic yard (~1.5 ton) of contaminated soil to incinerate was about \$500 to \$1000 depending on other conditions (Mr. Charles Lechner, U.S. Army AEC, Aberdeen Proving Ground, MD, personal communication, EPA, 1990). In order to facilitate compliance with EPA regulations, the U.S. Army initiated the program to develop toxicity data on TNB to develop a RfD, which can be used to develop environmental and health effects criteria for clean up and remediation.

METHODS

Chemical: 1,3,5-trinitrobenzene CAS# 99-35-4, (99.83%) was prepared by Dr. W.Koppes, Naval Surface Warfare Center, Silver Spring, MD, and the purity of the compound was further confirmed by HPLC by the Army and EPA (Reddy et al., 1993).

Experimental: All studies, acute, subchronic, chronic toxicity, toxicokinetics, reproductive and developmental toxicity, were conducted using TNB prepared from the same batch. These studies were conducted according to standard EPA health effects testing guidelines (40CFR 798) in compliance with GLP (40CFR 792). Toxicokinetics (absorption, distribution and elimination) of ^{14}C -TNB was studied in Fischer 344 rats following a single oral dose (Reddy and Gunnarson, 1993). Rats were dosed with ^{14}C -TNB (152 mg/kg; 6 to 8 mCi) in dimethyl sulfoxide and placed in glass

metabolism cages. ^{14}C -TNB levels in urine and feces were measured at 24, 48, 72 and 96 hr after dosing. The expired ^{14}C - CO_2 was measured for 48 hr. Tissue samples were taken at termination of experiments (after 4 days).

RESULTS

Acute toxicity

Acute toxicity tests with TNB were conducted by FitzGerald et al., (1992 a). They showed that TNB is not a dermal irritant and did not produce dermal toxicity (2 g/kg), but showed severe eye irritation potentials in rabbits and mild skin sensitization in guinea pigs. The acute oral LD50 values in male, female and combined sexes of F344 rats were 298, 275 and 284 mg/kg respectively. The oral LD50 values (mg/kg) for mice were >900 for male, 702 for female and 804 for combined sexes. Mice appear to be less sensitive than rats. These values are comparable to the oral LD50 values (mg/kg) of 450 for white rats, 600 for white mice and 730 for guinea pigs (Korolev et al., 1977) . The toxicity to these animals was characterized by central nervous system and respiratory disorders and cyanosis. Timofievskaya and Rodionova (1973) also reported an oral LD50 of 572 mg/kg in mice. No details are reported in these abstracts published in Russian.

Toxicokinetics and Metabolism

Toxicokinetics: The toxicokinetics (absorption, distribution and elimination) results showed about

10% of the dose (^{14}C -TNB, 152 mg/kg; 6 to 8 mCi) was eliminated in urine of male and female rats in the first 24 hr. About 21% and 36% of the dose appeared in the urine in male and female rats in 4 days, respectively. Excretion via feces was about 4% in the same period in both sexes. The expired ^{14}C - CO_2 comprised about 3% and 5 % of the dose over 2 days in male and female rats respectively. Soft tissue analysis 4 days after treatment revealed the radioactive residues of about 0.02 to 0.03%/g in liver, kidney, skin, and lungs, whereas other tissues showed substantially lower levels of residues $\leq 0.001\%$ /g. The results showed that a single oral dose of TNB in the rat was absorbed in the gut and was eliminated mainly in urine, with low levels in feces in 4 days. These results also show low levels of TNB residues in tissues examined after 4 days.

Metabolism: Bel et al., (1994) determined TNB and its metabolites in biological fluids of Sprague-Dawley rats fed diet containing TNB and identified reductive metabolites, 3,5-dinitro aniline (urine), 3-amino-5-nitroaniline (urine, feces and blood) and 1,3,5-triaminobenzene (urine and feces). No TNB was found in the samples by a GC/MS method. No details of dose, time were provided in the abstract. Reddy et al., (1996) studied the metabolism of ^{14}C -TNB (43 μg) in an *in vitro* rat liver microsomal system and observed that TNB is completely metabolized (within 5 minutes) and subsequently identified two metabolites as 3-amino-5-nitroaniline and 3,5-dinitro aniline by either spiking or coelution with authentic standards by HPLC. At least one additional, unidentified metabolite was also observed. These results suggest that TNB is metabolized in *in vivo* and *in vitro* systems.

Subacute and subchronic toxicity

Subacute Toxicity

The subacute (14-day) toxicity of TNB was evaluated in male and female F344 rats (100 to 120 g; 8 weeks old) by feeding diet containing TNB at dose levels of 0, 50, 200, 400, 800, and 1200 mg/kg diet (Reddy et al., 1994 a; Reddy et al., 1996). The calculated average TNB intake for male rats was 4.41, 17.08, 34.30, 55.87 and 92.33 mg/kg body weight/day and for female rats 4.42, 17.72, 34.26, 59.19 and 79.68 mg/kg body weight/day. These studies were conducted to select suitable doses for subsequent subchronic (90-day) toxicity studies. The mean daily food consumption significantly decreased in female rats fed 34.2, 59.2 and 80 mg/kg/day and in male rats fed 56 and 92.3 mg/kg/day. Average daily water consumption was decreased in males fed high dose (56 and 92.3 mg/kg/day). This decreased food intake and water consumption resulted in reduced body weight in the 92.3 mg/kg/day dose group.

Mean ratio of relative organ weights to final body weight showed significant changes in both sexes of rat. Mean relative brain weight (1200 mg/kg diet dose group) and spleen weight (400, 800 and 1200 mg/kg diet dose) for both sexes were significantly increased, while the relative thymus weight was significantly decreased in male rats (1200 mg/kg diet). In male the relative testicular weight was decreased significantly in rats fed 800 and 1200 mg/kg diet, while weights of kidneys increased significantly in male rats fed 200 to 1200 mg/kg diet. The relative liver weight was increased in the 400 mg/kg group of males but decreased in the 50 mg/kg diet female group.

Hematology and clinical chemistry results showed a decrease in red blood cell count, hematocrit and hemoglobin levels and decrease in alkaline phosphatase levels and increase in methemoglobin concentrations as compared to control dose related. There were no biologically significant changes of any analytes except for significantly decreased alkaline phosphatase levels. Histopathological analysis showed that TNB produced significant changes in the testis (seminiferous tubular degeneration), spleen (extramedullary hematopoiesis), and brain (hemorrhage and malacia) in 400 to 1200 mg/kg group. The kidneys of male rats in the 200, 400, 800 and 1200 mg/kg dose groups exhibited an increased incidence of cortical tubular hyaline droplet deposition.

Subchronic Toxicity

Rats: Subchronic (90-day) oral toxicity studies of TNB in male and female Fischer 344 rats (120 to 130 g, 8 weeks old) were conducted by Reddy et al., (1994 b, c). Rats were fed a diet containing 0, 66.67, 400 and 800 mg TNB/kg diet for 90 days. The average daily TNB consumption was for females 4.3, 24.7 and 49.3 and for males 3.9, 22.7 and 44.2 mg/kg/day. Male and female rats receiving 400 and 800 mg/kg diet consumed less, which resulted in significant decrease in body weight. However the water consumption in females in those groups was significantly increased. The mean relative organ weights (g/100 g BW), liver and spleen weights (males and females) and brain weights (males) were increased significantly in rats receiving 400 and 800 mg/kg diet. The relative testicular weight was decreased significantly in males.

Subchronic exposure to TNB produced hematological effects in rats . A significant decrease in total red cell count in male (22.7 and 44.2 mg/kg/day) and female (24.7 and 49.3 mg/kg/day) was noted. Correspondingly, there was a significant increase in the percent of reticulocytes in males (22.7 and 44.2 mg/kg/day) and in all female groups. A decrease in hemoglobin content and a significant increase in methemoglobin was observed in both mid and high dose groups of males and females (400 and 800 mg/kg diet). Clinical chemistry results showed no significant changes in any of the analytes studied.

Histopathological analysis revealed a moderate to severe seminiferous tubular degeneration in testis of mid and high dose groups (22.7 and 44.2 mg/kg/day) and deposition of hyaline droplets in kidneys of all male rats receiving TNB diet. The spleen and bone marrow featured mild to moderate erythroid cell hyperplasia in both sexes of rats receiving mid and high dose group (400 and 800 mg/kg diet).

Male rats receiving TNB at dose levels of 3.9, 22.7 and 44.2 mg/kg/day had an increased incidence of cytoplasmic droplets (hyaline droplets) in proximal cortical tubular epithelial cells of the kidney at all treatment levels. The severity of this change was dose-dependent, ranging from moderate in the high dose group (44.2 mg/kg/day) to mild in the low dose group (3.9 mg/kg/day). These droplets were occasionally irregularly shaped and had angular contours but were more often spheroid. They stained positive with Mallory-Heidenhain protein stain. Further characterization of these droplets would require immunohistochemical staining. A diagnosis of alpha-2-u-globulin nephropathy was not deemed appropriate since there was no significant increase in single cell necrosis, no presence of granular casts or linear papillary mineralization or increased tubular hyperplasia. In addition to the

deposition of hyaline droplets, the presence of early chronic progressive nephropathy was evident in both treated as well as control male rats. This change was characterized by an increased incidence of tubular degeneration and regeneration as well as mineralized foci. Tubular degeneration was the only change which appeared to be dose related as noted by an increased severity (mild) in the high and mid dose groups.

From these subchronic toxicity studies, a No Observed Adverse Effect Level (NOAEL) of 4.3 mg TNB/kg/day was established for female rats. Because TNB produced toxic effects in the male kidney (deposition of hyaline droplets) at all doses tested, Low Observed Adverse Effect Level (LOAEL) of 3.9 mg/kg/day is suggested.

White-footed mice: Subchronic (90-day) toxicity of TNB was evaluated in the white-footed mouse (Peromyscus leucopus), of 8 to 12 weeks old (Reddy et al., 1994 d). Animals of both sexes were fed diet containing 0, 150, 375 and 750 mg/kg diet. The average calculated TNB consumption was 20.16, 64.81, and 108.25 mg/kg/day for females and 23.50, 67.44 and 113.51 mg/kg/day for males. The only significant organ changes noted were increase in relative kidney weight (females) and in an absolute and relative spleen weight (male) mice of the 750 mg/kg group. Hematology analysis showed no significant changes in the females while the males presented a significant increase in reticulocytes at 150 and 750 mg/kg diet groups and increase white blood cells in the 750 mg/kg group. Histopathological analysis revealed treatment related changes in spleen (erythroid cell hyperplasia) and in testis (seminiferous tubule degeneration) in the 750 mg/kg diet group. The only biologically findings (histopathological changes) deemed significant were those present in the male

750 mg/kg group. From this subchronic toxicity study, a NOAEL of 20.1 mg/kg/day for female and 23.5 mg/kg/day for male mice is suggested. These results also show that the white-footed mouse is less sensitive to TNB than rats. The target organ toxicity profile for this species was similar to that observed in rats but at higher dose.

Reproductive Toxicity

A modified Screening Information Data Set (SIDS) for a single generation reproductive toxicity study of TNB was conducted in male and female Sprague-Dawley rats (Kinkead et al., 1994, 1995). Rats were fed a diet containing 30, 150 and 300 mg of TNB/kg diet. The calculated dose consumed was 2, 9 and 19 mg/kg/day for males and 3, 14 and 29 mg/kg/day for females. Male rats received TNB from 14 days prior to the mating and throughout the mating period for a total of 28 days. Female rats were exposed to TNB from 14 days prior to mating and during the mating, gestation, postpartum (21 days) periods and also 4 weeks postweaning for a total of 90 days. Pups were maintained on treated diet through 4 weeks post weaning.

No mortality occurred in the parental animals during the study and no treatment related differences were noted in absolute or relative organ weights in male rats necropsied following the mating period (28 days of treatment). However an increase in absolute and relative spleen weight (300 mg/kg), relative liver weight (300 mg/kg) and relative kidney weight (150 and 300 mg/kg) was observed in females following 90 days of exposure. Male rats sacrificed following 28 days mating showed sperm effects including reduced number and concentration of motile spermatozoa (300 mg/kg) and reduced

percent of cells showing a circular motion pattern (150, 300 mg/kg). Histopathological evaluation revealed significant increase in splenic hemosiderosis (150 and 300 mg/kg) and presence of hyaline droplets (mild) in control and all treated animals after 28 days of treatment, which may not be treatment related.

TNB showed no adverse effects on reproductive indices, on mating (100%) or fertility index (92% in 300 mg/kg and 100% in other groups). No significant treatment related differences were noted in length of gestation, sex ratio, gestation index, or mean number of offspring per litter. During the 21-day lactation phase, the mean body weights of the TNB-treated pups (male and female) were significantly less than the control group pups, except at the 14 days time point when the 150 and 30 mg/g group pups were equal to control. These results showed that TNB produced organ toxicity at 150 and 300 mg/kg dose (males) but no adverse effects on reproductive indices in any dose groups. The calculated NOAELs based reproductive toxicity end points such as reproductive indices (mating, fertility and other given above) were 2 mg/kg/day for males, and 3 mg/kg/day for females.

Developmental Toxicity

Developmental toxicity of TNB (in 1% agar in sterile water) in female rats (Sprague-Dawley Crl:CD BR) was studied by oral gavage at dose levels 11.25, 22.5, 45.0 and 90.0 mg/kg/day (Cooper and Caldwell 1996). Animals were identified as sperm positive on day 0 and were given TNB on gestation days 6 through 15. A laparohysterectomy was performed on all surviving animals on gestation day 20. Evidence of maternal toxicity was expressed at a dose level of 90 mg/kg/day by

significant decreases in body weight and in food consumption. Clinical changes consistent with toxicity included disorientation, shaking, unsteadiness and hyperactivity observed in one animal (1/25) in the 90 mg/kg/day group. Developmental toxicity exhibited in the 90 mg/kg/day group included reduced mean fetal weight and crown-rump length and increased incidence of skeletal variation (in one animal). No maternal toxicity or developmental toxicity was observed at dose levels of 11.25, 22.50 and 45.00 mg/kg/day. Based on this study, a dose level of 45 mg/kg/day was considered to be the NOAEL for developmental toxicity.

Mutagenicity

Evaluation of available literature revealed that TNB is mutagenic in Salmonella typhimurum strains (McGregor et al., 1980; Spanggord et al., 1982; Kawai et al., 1987). However TNB did not appear genotoxic in the DNA repair assay with Escherichia coli or in the mitotic recombination assays with Saccharomyces cerevisiae D5 (McGregor et al., 1980).

Chronic Toxicity/Carcinogenicity

Slaga et al., (1985) studied the carcinogenic activity of TNB in mouse skin and lung tumor assays. A single application of 10 or 50 mg of TNB (in acetone) to the skin of mice increased the incidence of inflammation, epidermal hyperplasia and dark cells. The response elicited by these dose levels was similar to the maximum response obtained with 12-O-tetra decanoyl phorbol 13-acetate (TPA) (a potent promoter of two-stage carcinogenic tumors in the skin of SENCAR strain mice). However TNB was tested negative in a skin initiation assay which employed TPA-promotion for tumor

expression. Górski, (1969) studied the biological role of charge transfer complexes of aromatic hydrocarbon oxi-derivatives in chemical carcinogenesis in male BALB/c strain mice . He injected subcutaneously 3-methyl cholanthrene (3MC), its complex forms with TNB and TNB itself (equivalent to 1 mg of 3MC) in 0.4 ml paraffin oil. The charge transfer complex from the oxidation product of 3MC with TNB revealed* carcinogenic activity on mice higher than for 3MC by itself. Animals receiving TNB (1 mg equivalent of 3MC) alone were in good condition and remained without tumors throughout the observation period (144 days). This showed that TNB alone is not tumorigenic in mice in this study.

Chronic (2- year) toxicity study of TNB was conducted in male and female F344 rats (Reddy et al., 1996). Rats were fed diet supplemented with TNB, 0, 5, 60, and 300 mg/kg diet and were sacrificed at 3, 6, 12, and 24 months after exposure. The calculated average TNB consumption over the 2-year exposure period at different time periods is presented in table 1. The calculated average TNB consumption at 2-years for females was 0.23, 2.68 and 13.31 mg/kg/day and 0.22, 2.64 and 13.44 mg/kg/day for males. The body weight of both sexes decreased in the high dose (300 mg/kg) groups. The relative spleen weights were decreased in both sexes while brain weights were increased in females (300 mg/kg group). Hemoglobin and hematocrit levels were decreased in both sexes in the 300 mg/kg group during 12 months (3, 6, 12 months) but no significant difference was evident at the end of the study (24 months). However, methemoglobin was increased in females (300 and 60 mg/kg groups at 12 months) and in both sexes (300 mg/kg at 24 months). Thus based on this study histopathological examinations suggested that the susceptible organs for TNB toxicity were kidneys (cytoplasmic droplets) in both sexes, spleen (erythroid cell hyperplasia/pigment deposition)

in both sexes of 60 and 300 mg/kg diet dose groups and testis (seminiferous tubular degeneration) in the males treated with 300 mg/kg. During this period no other lesions related to carcinogenic activity were detected. The results of final study (2-year) indicated toxicological events, such as methemoglobinemia, kidney and testicular lesions due to TNB exposure in both sexes of rats. These toxic effects were also observed in the interim (1-year) sacrifice but severity was significant only in the high dose (300 mg/kg). The lesions observed in various organs were nonspecific and unrelated to TNB. From this chronic study a NOAEL of 2.64 mg/kg/day and a LOAEL of 13.31 mg/kg/day for both sexes of rats are suggested.

Structure-activity relationships

Previous studies have shown that the nitroaromatic compounds such as nitrobenzene (NB), DNB and TNT produced hematological effects (methemoglobinemia, anemia), testicular degeneration, reproductive, and CNS effects in animals (Bond et al., 1981, Cody et al., 1981, Levine et al., 1984, Morgan et al., 1985; Philbert et al., 1987). The oral LD50 values for combined sexes of rats were 59.5 mg/kg and 284 mg/kg for DNB and TNB, respectively. (Fitzgerald et al., 1992 a, b). The additional nitro group resulted in reduced toxicity. Comparative acute toxicological data indicate that DNB is considerably more potent than TNB. Since there was no toxicity data on TNB, EPA had developed RfD on the basis of the subchronic toxicity on DNB (Cody et al., 1981). In this study a LOAEL was calculated based on increased spleen weights in Carworth Farm male rats treated with 8 mg/L (1.13 mg/kg/day) DNB. The corresponding NOAEL and LOAEL for TNB (mg/kg/day) were determined by multiplying the NOAEL/LOAEL of DNB by the molecular weight ratio of TNB/DNB

(213.11/168.11=1.27). In the subsequent risk estimates (e.g. Derivation of RfD) the uncertainty factors of 10,000 (including a factor of 10 for subchronic to chronic exposure, 10 for interspecies extrapolation, 10 for sensitive members of the human population, and 10 for the derivation of RfD by analogy to structurally similar DNB).

Thus, the RfD for TNB, based on DNB toxicity was calculated as follows:

NOAEL	3 mg/L DNB in drinking water, converted to 0.51 mg/kg/day TNB
Uncertainty factor:	10,000
Modifying factor	1
Chronic oral RfD:	5E-5 mg/kg/day

Interpretation of data

The U.S. Army conducted several animal toxicity studies discussed above which have demonstrated adverse health effects (hematological and testicular) of TNB at high doses. The NOAEL and LOAELs for subchronic, reproductive and developmental toxicities were estimated (see above). The subsequent two year toxicity studies conducted in F344 rats showed that TNB produced hematological, testicular, and kidney effects in rats exposed at high doses (circa 13 mg/kg/day) from 3 to 24 months. The hematological effects such as methemoglobinemia were not exhibited at 24 months suggesting the animals may have compensated for these effects over the longer term. TNB effects on the kidney were observed at the 3, 6, 12, and 24 months time points (Reddy et al., 1996).

These kidney effects are accumulation of cytoplasmic hyaline (protein) droplets in proximal tubule in male rats exposed to 2.64 or 13.44 mg/kg/day. These effects were also observed in female rats at 12 and 24 months but these effects were not observed in female earlier at 3 or 6 months during the study. Immunohistochemical analysis of kidneys of rats exposed to TNB revealed the presence of alpha-2 μ -globulin in some, but not all hyaline droplets formed in proximal tubular epithelium in both sexes. Those droplets, which were treatment-related did not demonstrate strong positive staining for alpha-2 μ -globulin in female rats. Older female rats (104-week) may exhibit some characteristics associated with males due to age related hormonal changes. The histopathologic and immunohistochemical findings from the present study (Reddy et al., 1996) do not support the histopathology and lesion progression observed in typical reports of alpha-2 μ -globulin associated nephrotoxicity. Furthermore, in the present study, there was no increase in response to TNB treatment. Thus the pathological sequence of lesions associated with alpha-2 μ -globulin nephropathy were not observed as described in EPA Risk Assessment Forum Guidelines (1991). The typical lesions include single-cell necrosis, exfoliation of epithelial cells into the proximal tubular lumen, formation of granular casts, linear mineralization of papillary tubules and tubule hyperplasia. These lesions were not observed in the present study; therefore, these results preclude consideration of TNB-nephrotoxicity for this risk assessment.

Hyaline (protein) droplet accumulation in proximal tubules has been observed in male rats exposed to unleaded gasoline, 2,2,4-trimethylpentane, decaline, 1,4-dichlorobenzene, pentachlorobenzene and d-limonene, the natural product found in citrus oils (Hard et al., 1993). This protein is not found in humans. TNB showed mutagenicity in a bacterial system but in short term carcinogenic bioassay and

in the chronic 2-year studies (described above) revealed no carcinogenicity. None of the lesions observed in the 2-year study appear to be attributable to TNB treatment. Therefore the doses 2.64 and 13.31 mg/kg/day are considered as NOAEL and LOAEL, respectively for the derivation of an RfD in the present study. Thus based on TNB toxicity studies (as outlined above) the following approach is proposed for calculation of the TNB RfD:

Derivation of RfD

NOAEL:	2.68 mg/kg/day (Chronic toxicity studies of TNB)
Uncertainty Factor:	100
Modifying Factor:	1
Oral RfD:	3E-2 mg/kg/day

Data obtained from the chronic study clearly demonstrated TNB-induced toxicity to the hematopoietic system. Such effects have also as has been demonstrated for other nitroaromatics such as dinitro benzene and trinitrobenzene. Application of an uncertainty factor of 100 (10 for animal to human extrapolations and 10 for human sensitive population) to the NOAEL of 2.68 mg/kg/day results in an RfD of 3E-2 mg/kg/day (as shown above). It is worth noting that the new RfD (0.03 mg/kg/day) is 600 times higher than the previously estimated RfD (0.00005 mg/kg/day) (based on DNB toxicity data). The uncertainty factors used in this were only 100, as the toxicity data obtained from TNB per se of chronic toxicity study, increased the confidence in the toxicological data base. These studies are conducted under full GLP standards and contain considerable detail as (e.g.,

animals were evaluated at 3, 6, 12 and 24 months). These studies are further supported by the subchronic toxicity studies in F344 rats and in white-footed mice (second species), and with additional subchronic reproductive and developmental toxicity studies. Higher confidence is recommended for these data. Furthermore the absence of neoplastic lesions in chronic toxicity studies and the kidney effects (alpha 2 μ -globulins) observed in rats are considered not to be relevant for humans in the risk assessment.

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TABLE 1**Estimated TNB Consumption**

Exposure Duration (Months)	Female			Male		
	mg TNB/kg diet					
	5	60	300	5	60	300
	mg TNB/kg/day					
3	0.27	3.21	15.17	0.35	3.72	17.93
6	0.27	3.23	14.37	0.28	3.22	15.69
12	0.24	2.93	14.90	0.25	2.96	14.61
24	0.23	2.68	13.31	0.22	2.64	13.44

**ABSTRACT OF PRESENTATION:
TRICHLOROETHYLENE: WHAT ARE
THE APPROPRIATE STANDARDS?**

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Trichloroethylene (TCE), a chlorinated solvent commonly used in the past for degreasing metal parts, is second only to Total Petroleum Hydrocarbons as an environmental contaminant within the Air Force (AF). During the period of the 1940s through the 1960s, it was common practice to dispose of TCE by dumping in unlined landfills or simply by pouring it on the ground. Presently, nearly 1700 TCE contaminated sites at 284 installations have been characterized throughout Department of Defense (DOD). In addition, there appears to be an almost one to one correlation between installations with any media contaminated with TCE and installations with TCE contaminated groundwater.

In the early 1970s, studies began to suggest that TCE could be a carcinogen. In 1975, the National Cancer Institute designated TCE as a suspect carcinogen. Based on this designation, the Food and Drug Administration banned TCE for use in food processing. The first Health Assessment Document (HAD) on TCE was published in 1985 by the U.S. Environmental Protection Agency (EPA). In 1987 the HAD was updated to include a carcinogenicity classification for TCE. Based on the carcinogen classification, EPA's Office of Water set a maximum contaminant limit goal (MCLG) for TCE at 0. The regulated standard, maximum contaminant limit, or MCL, was set, by policy, as close to the MCLG as economically and technologically feasible. The current federal standard for drinking water is 5 ppb and is often used as a remediation goal for groundwater. The most commonly used remediation

technology for TCE contaminated groundwater is pump-and-treat. Questions remain as to whether pump and treat technology can achieve the 5 ppb drinking water standard.

The Air Force position is that it is time to revisit the TCE HAD with the goal of bringing all the current science to the table for the development of risk based health standards. Through multiagency cooperative groups (the Environmental Risk Assessment Program and DOD Trichloroethylene Working Group), DOD has committed to partnering with EPA in revisiting both the standards for noncancer and cancer effects. Although there is much new science to bear on the process, along with increased flexibility for analyzing the data within the new Cancer Guidelines, the controversy surrounding the carcinogenicity of TCE is far from being resolved. This is reflected by carcinogenicity classifications ranging from "not suspected as a human carcinogen (American Conference of Governmental Industrial Hygienists)" to "probably carcinogenic to humans (International Agency for Research on Cancer)". Uncertainty remains regarding the appropriate animal species and strain, appropriate cancer endpoint, and even what are the pertinent metabolic pathways for extrapolating risk values to humans. Much of the uncertainty revolves around the mechanism of action for specific tumors in the different animal models.

Despite the problems surrounding TCE and risk assessments, the efforts to review the current state of the science and to appropriately revise the current standards to incorporate the most recent data in terms of the new Cancer Guidelines has provided an opportunity for DOD and the EPA to establish a closer working relationship. Exactly what the standards will be is yet to be determined, but the risk assessment community continue to refine the risk assessment process to provide improved

standards based on the current knowledge of mechanisms of action and metabolic pathway analyses appropriate for human extrapolation. This cooperative effort between DOD and the EPA to resolve the controversy over safe standards for TCE may provide a template for other cooperative efforts between DOD and other U.S. agencies.

**ABSTRACT OF PRESENTATION:
AN EVALUATION OF THE EFFECTS OF
IONIZING RADIATION ON TERRESTRIAL
BIOTA: IMPLICATIONS FOR THEIR
PROTECTION**

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The DOE Division of Air, Water, and Radiation (EH-412) is preparing to issue protective radiological standards for aquatic and terrestrial organisms. To support this effort, the DOE sponsored a workshop to evaluate the adequacy of current approaches. Workshop participants reviewed and discussed a 1992 International Atomic Energy Agency (IAEA) report on radiological protection of biota for its adequacy and completeness in answering the following question: Can these data and conclusions be used for promulgating radiological standards for the protection of terrestrial organisms? Are the conclusions given in this report still valid or have they been superseded by more recent data?

The consensus from the workshop was that the dose limits for animals and plants recommended by the IAEA are adequately supported by the available scientific information. Participants agreed, however, that better guidance on application of those limits is needed. Participants further agreed with the IAEA that protecting man generally protects biota, except when (1) human access is restricted, (2) unique exposure pathways exist, (3) rare or endangered species are present, or (4) other stresses are significant. To deal with these exceptions, site-specific analyses of exposures should be considered in developing secondary standards.

**ABSTRACT OF PRESENTATION:
HUMAN HEALTH RISK ASSESSMENT
FOR PETROLEUM HYDROCARBON
CONTAMINATED SITES**

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The evaluation and remediation of petroleum hydrocarbon impacted sites is difficult because of the complexity of scientific, regulatory, and resource issues surrounding this problem. Many states use levels of total petroleum hydrocarbons (TPH) to initiate remedial actions and set cleanup levels. The Total Petroleum Hydrocarbon Criteria Working Group was formed to determine health risk-based alternatives for the evaluation of petroleum hydrocarbon impacted sites. The projects under way in the group are focused along four technical areas: analytical chemistry, toxicology, and fate and transport, risk-based corrective action. This presentation will discuss the status of the projects completed to date and what future expectations are for demonstration of the Group's protocol and interaction with state regulatory agencies.

Analytical: The projects include: "Summary Composition of Petroleum" and a primer on "TPH Analysis in Soil and Water". The first is designed to identify the critical components in petroleum products. Without knowing what is in the fuel it is very difficult to assess the potential risk the mixture poses. The primer is a reference tool to explain the complexity of analytical procedures used to evaluate fuel contaminated soils.

Fate and Transport: The main effort from this group has been the review of critical fate and transport parameters for petroleum components. The components are grouped into fractions based on order of magnitude variation in mobility in the environmental media. This project is designed to answer

the question of whether the components move in the environment or stay put. It also creates the groupings of compounds from which the chemical surrogates will be selected.

Toxicology: Based on fraction specific fate and transport information, toxicity information for the compounds in each fraction have been identified as surrogate compounds.

Risk-Based Framework: This group is working to bring the technical information together in an integrated risk-based approach. The method will include an assessment of hazard using the surrogate toxicity criteria, exposure using the surrogate physical/chemical parameters, and then an integration into the risk paradigm.

**ABSTRACT OF PRESENTATION:
OVERVIEW OF THE ROCKET
EMISSIONS WORKING GROUP (REWG)**

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Since 1992, Armstrong Lab has been helping Air Force east and west coast launch centers on issues pertaining to human health risks associated with exposure to emissions from rocket launches. The launch centers had originally proposed a three-tiered approach to exposure and safety. In response to a renewed request for more comprehensive guidance from Space Command's Surgeon General and the range safety offices at both launch centers, AL/OEMH organized the Rocket Emissions Work Group. REWG was tasked to provide Space Command with current, legally and scientifically defensible recommendations for health-based exposure standards governing acceptable criteria for potential public exposure to emissions from space rocket operations. The recommendations would be used as endpoints for the exposure/response functions to be incorporated into the "Launch Area Toxic Risk Analysis" model, a prospective tool to help launch commanders make go/no-go decisions. REWG drew its membership from the regulatory, military/space command, modeling, toxicology, and risk assessment communities and examined the available literature on rocket exhaust components. It generated interim consensus recommendations to the Space Command's Surgeon General on HC1, Nox, and HNO3. These interim standards are being forwarded to the National Research Council's Committee on Toxicology for peer review.

SESSION 11
ECOLOGICAL RISK: ASSESSMENT, INCORPORATION,
AND APPLICATION

1

**ABSTRACT OF PRESENTATION:
TRI-SERVICE COORDINATION OF
ECOLOGICAL RISK ASSESSMENT
ACTIVITIES**

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For the past year, tri-service scientists interested in ecological risk assessment have been meeting to discuss methodologies, procedures, and regulatory guidance. The pooling of technical expertise has benefited each service. The paper will present current activities, the development of tri-service guidance, and methods to conduct environmental audits. The tri-service scientists involved in these activities are from the following organizations: U.S. Army Center for Health Promotion and Preventive Medicine; U.S. Army Edgewood Research, Development, and Engineering Center; U.S. Army Corps of Engineers Waterways Experiment Station; U.S. Army Environmental Center; U.S. Air Force Armstrong Laboratory; Air Force Center for Environmental Excellence; and U.S. Navy Northern Division, Naval Facilities Engineering Command.

**ARMY CORPS ECOLOGICAL RISK ASSESSMENT GUIDANCE
DOCUMENT**

(EM-200-1-4, Risk Assessment Handbook, Volume II: Environmental Evaluation)

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ABSTRACT:

The U.S. Army Corps of Engineers (USACE) guidance for ecological risk assessment (ERA), EM 200-1-4, ***Risk Assessment Handbook, Volume II: Environmental Evaluation***, is now available. With the assistance of Woodward-Clyde Consultants, Hazardous, Toxic, and Radioactive Waste (HTRW) Center of Expertise (CX) risk assessor Terry Walker has developed a comprehensive document that addresses ecological risk assessment from a Corps perspective, focusing on site closeout. A companion to ***Risk Assessment Handbook, Volume I: Human Health Evaluation***, the document addresses the regulatory background requirements, scoping considerations, evaluation of Architect-Engineer (A-E) prepared risk assessments, and appraisal of risk management issues.

Built upon the United States Environmental Protection Agency's (USEPA) ***Framework For Ecological Risk Assessment*** (EPA/630/R-92/001), the document maintains compatibility with other current ecological guidance, including the recently published ***Procedural Guidelines for Ecological Risk Assessments at U.S. Army Sites***, (ERDEC-TR-221), written by the U.S. Army Edgewood Research, Development and Engineering Center (USAERDEC). Additionally, extensive references to both written and electronic media are provided, allowing the user to access the most up to date ecological information available.

BACKGROUND

The United States Army Corps of Engineers (USACE) guidance for Ecological Risk Assessment (ERA) is now available. As the USACE typically conducts environmental restoration investigations utilizing Architect-Engineer (A-E) contractors, a document detailing the regulatory process, scoping considerations, reporting format and content, and available references was needed. Built upon the United States Environmental Protection Agency's (USEPA) *Framework For Ecological Risk Assessment*, (EPA/630/R-92/001), the document maintains compatibility with other current guidance, especially the recently published *Procedural Guidelines For Ecological Risk Assessment At U.S. Army Sites*, (ERDEC-TR-221). The new document, *Risk Assessment Handbook, Volume II: Environmental Evaluation*, (EM 200-1-4), is a companion to the USACE guidance *Risk Assessment Handbook, Volume I: Human Health Evaluation*.

ERA SCOPING CONSIDERATIONS

Establishing the Level of Effort - EM 200-1-2, *Technical Project Planning Guidance for HTRW Data Quality Design*.

The USACE recognizes the need for cost-effective and efficient site investigation/response actions. The HTRW Engineer Manual (EM), *Technical Project Planning Guidance for HTRW Data Quality Design*, provides guidance on data collection programs and defines the Data Quality Objectives (DQOs) for HTRW sites. The HTRW technical project planning (TPP) process is a four-phased approach that begins with the development of a site strategy and ends with the selection of data collection options.

Project planning includes a review of the available background material and discussions to define the scope and critical aspects of the ERA. Spatial boundaries such as the size of the site, extent of contamination, potential threats to onsite and nearby ecosystems, and important ecosystem components (e.g., fisheries) greatly determine the potential scope and design of the ERA.

The purpose of an ERA is not to prove an ecological effect or accurately predict such effect, but to reasonably determine the degree to which hazardous constituents or wastes have impacted or could impact the structure, function, and dynamics of the ecosystems (i.e., biological diversity, functional integrity, energy and nutrient dynamics).

PHASE I - DEVELOP PROJECT STRATEGY

This phase of TPP involves identifying site decision requirements and developing an approach to address these requirements. The risk assessor is crucial to the development of

appropriate site strategy in this phase and the identification of data needs/quality to support risk management decisions. In this planning phase, site conditions are reviewed qualitatively, and a preliminary Ecological Conceptual Site Model (ECSM) is developed to help define the study elements for the current and subsequent project planning phases.

PHASE II - IDENTIFY POTENTIAL DATA NEEDS TO SUPPORT DECISIONS

This phase of the TPP process focuses on identifying data needs and minimum data quality requirements to support site decisions. Data users identify potential data needs and their respective proposed quality assurance/quality control requirements based on site background, regulatory information, and the customer's goal. At this phase, the compliance specialist, remedy-design engineer, and responsibility-legal data users, who have specific data needs, present their data requirements along with the data needs identified by the risk assessor. The objective is to identify all data needs and quality requirements of all project team members.

PHASE III - IDENTIFY DATA COLLECTION OPTIONS

This phase of the TPP process incorporates previously identified data needs and project constraints in designing a data acquisition approach. It also involves identifying the optimum sampling/data collection scheme so as to minimize mobilization, field sampling, and demobilization efforts and costs. The objective of Phase III is to identify options (preferably two or three options, out of which one is an optimum option) for presentation in Phase IV.

PHASE IV - SELECT DATA COLLECTION OPTIONS AND ASSIGN DQOs

This is the most important phase of the project planning/execution process, because this is where data collection options are selected. The product of this phase of the TPP process is the Statement of Work (SOW) for USACE work acquisition, a detailed cost estimate for the selected option, and DQOs for the data collection program.

The Phase IV project planning process involves not only the selection of the data collection program in support of an ERA or risk analysis, but also documents the reasoning for the selections. Such documentation will provide a historical knowledge which justifies and guides the data collection, review and data use.

INTRODUCTION TO THE FOUR-TIERED APPROACH

This handbook incorporates a four-tiered approach to the

conduct of a baseline ERA and the evaluation of potential adverse effects on ecological receptors. The tiered approach to the baseline ERA is composed of sequentially more sophisticated and complex evaluations. Therefore, scoping of the ERA for different tiers will require various data needs to be satisfied. Sequential evaluation, feedback, and flexibility allow for sound scientific judgements and efficient use of resources by minimizing unnecessary data collection, focusing major efforts, and optimizing benefits.

While the tiered approach is intended to maximize efficiency of data collection, there are cases where the tiered approach may require multiple field programs or time delays. In some cases, logistics and cost considerations outweigh the benefits of tiered testing.

TIER I - PRELIMINARY ECOLOGICAL RISK ASSESSMENT

The Tier I ERA is characterized by relatively simple, quantitative wherever possible, desk-top methods that rely heavily on literature information, previously collected data, and a chemical-concentration based approach.

The Tier I ERA emphasizes adverse effects to the individual based on literature-cited toxicity values with extrapolations to potential impacts at the population, community, or ecosystem level. The Tier I ERA provides quantitative chemical information for the exposure point media (e.g., soils, sediments, surface water) and possibly qualitative biological data to fill gaps in the available data set. Field or laboratory bioassays are typically not part of a Tier I effort.

Development of a site-specific ECSM, selection of potential Contaminants of Ecological Concern (COECs), and a description of exposure pathways are major activities in this tier. Qualitative and quantitative data from a site reconnaissance or field survey of flora and fauna are summarized in an ecological site description. This field visit coupled with site-specific information provides for documentation of obvious adverse effects, identification of potentially important receptors, and development of simplified food web models to evaluate the potential for COECs to bioaccumulate in receptors of concern.

An initial site description is needed to orient the technical specialists. This information should be assembled from existing sources, without conducting formal field studies. Initially, base or facility natural resource personnel should be contacted as they often have relevant data or useful ecological information. Many state and federal agencies can provide information on sensitive areas or regional data on ecology, especially threatened and endangered species, checklists of biota, endemic species, and other pertinent ecological information. Local, regional, or university museums or state biological surveys may be other sources of information.

TIER II - FOCUSED BIOLOGICAL EVALUATION AND SAMPLING

The Tier II ERA is recommended where there is a need to reduce uncertainty or verify Tier I findings by using a biological effects-based, sampling approach. Proceeding to a Tier II, Tier III, or Tier IV ERA may also be necessary when field studies or bioassays are desired, when Tier I risk is not well-characterized, or when significant questions remain and remediation decisions cannot be adequately addressed. In Tier II, a shift is made to evaluating population and community level effects, as well as mixtures of chemicals and chronic effects using a biological effects-based approach. The overall objective in Tier II is to produce more accurate, quantitative predictions regarding current and future risks to ecological populations, communities, and ecosystems due to migration of chemicals from the contaminated site.

Tier II may include laboratory or field bioassays and/or more detailed, sophisticated computer models or probabilistic methods. Quantitative biological samples, as well as abiotic samples, as needed, may be collected to document exposure, to assess bioaccumulation potential, or to determine dose-response of the tested species or the selected receptors when exposed to site media. Tier II may include inexpensive, short-term toxicity tests or bioassays, standard rapid biological field assessment protocols, or focused tissue residue analyses of key receptors or their prey.

The biological sampling methods employed in Tier II are simple, short-term, and inexpensive relative to Tiers III and IV.

Tier II data, when integrated with data (primarily chemical) collected from the previous phases, should generally be adequate to provide information on the significance of potential or observed ecological effects, the need for remediation/removal actions, and the development of preliminary cleanup goals based on ecological concerns and remedial action objectives.

TIER III - EXPANDED SAMPLING PROGRAM

The Tier III ERA is recommended where longer-term or more extensive biological or chemical sampling programs are needed to resolve issues presented by larger sites having complex ecosystems and food webs. Depending on site conditions and complexity, elements of a Tier III ERA may be the most appropriate type of additional investigation following Tier I. The biological sampling conducted in Tier III may involve long-term (chronic) bioassays or tissue analysis of additional organisms or for additional analytes, and/or additional quantitative biological (i.e., population) sampling development. Additional ecosystem function or other field data may be collected, including nutrient loss (amount of undecomposed litter), biomarkers, histopathological examinations, or mesocosm studies (in-situ biomonitoring).

Results of the additional field and laboratory investigations fill the data gaps identified following completion of the previous tier (Tier II or I) and supplement the results from all studies conducted previously. The combined results are

used to present revised risk estimates with less uncertainty than the preceding tiers, or provide the rationale for long-term monitoring (Tier IV) if needed.

TIER IV - MONITORING PROGRAM

The Tier IV ERA is reserved for the largest and most complex sites and is only appropriate where multiple year, biological monitoring or sampling programs are needed, and an ERA with the highest degree of certainty is required.

Complex sites are those with chemical interactions among numerous COECs and exposure matrices, wide-spread contamination or numerous contamination sources, and sites requiring the examination of potential risk reduction over time. This tier includes biological studies of longer duration and greater expense (e.g., multi-year population and community level studies) or complex exposure modeling.

Tier IV investigations are expected to be warranted at very few sites.

EVALUATION OF CONTRACTOR-PREPARED ERAs

SCREENING LEVEL - Preliminary Assessment/Site Inspection/ RCRA Facility Assessment (PA/SI/RFA)

The screening ERA is a streamlined version of the complete *Framework* process and is intended to allow a rapid determination by the risk assessor and risk manager if the site poses no or negligible risk. The basis of the screening level assessment is the ecological site characterization and the comparison of site abiotic media concentrations with existing environmental criteria and guideline values (i.e. Applicable or Relevant and Appropriate Requirements, ARARs), such as federal and state Ambient Water Quality Criteria (AWQC); marine sediment effects levels (Long et al. 1995); freshwater sediment effects levels (Persaud et al. 1992); or other readily available screening-level ecotoxicity values.

Data available from PA/SI and RFA activities are usually limited in number but should be broad in scope of chemical analysis and in the number/type of abiotic media sampled.

Sampling should have been conducted in areas of suspected contamination and background areas to distinguish site contamination from background levels and to provide information on the "worst case." If sampling was not conducted in areas of suspected contamination, the screening ERA will not provide an adequately cautious assessment of potential risk. Similarly, if a broad chemical analysis was not performed, or if data are not available for all abiotic media of potential concern, the screening ERA will be limited and cannot be used to eliminate the site from further consideration.

For the screening ERA, assessment endpoints include any likely adverse ecological effects on ecological resources of

concern, for which exposure pathways are complete. Measurement endpoints are based on available toxicity values from the literature (i.e., toxicological endpoints).

In the absence of sound site-specific information, preliminary exposure estimates are usually based on conservative assumptions such as:

- Area use is 100 percent (for a particular habitat),
- Bioavailability is 100 percent,
- The most sensitive life stage is present, and
- Minimum body weight and maximum ingestion rate are used.

In general, if the 95% UCL or maximum chemical concentration exceeds the screening criterion, further assessment of the site is probably indicated.

BASELINE - Remedial Investigation/RCRA Facility Investigation (RI/RFI)

The decision to continue beyond the preliminary ecological risk screen does not indicate that risk is unacceptable or that risk reduction is necessary, rather it indicates that a more focused evaluation and characterization of the risk and accompanying uncertainty is needed.

The baseline ERA provides an objective, technical evaluation of the potential ecological impacts posed by a site. The baseline ERA should be clear about the approaches, assumptions, limitations and uncertainties in the evaluation, to enable the risk assessor and manager to interpret the results and conclusions appropriately.

EVALUATION OF REMEDIAL ALTERNATIVES - Feasibility Study/Corrective Measures Study (FS/CMS)

Various types of ERAs may be applied to conduct a screening evaluation of remedial alternatives or a more detailed analysis of a selected alternative. Generally, the Tier I baseline ERA will be sufficient in providing the risk inputs for selection of potential remedial alternatives or corrective measures (including the no-further action alternative) or the need for procedural changes or engineering controls to minimize short-term risks or residual risks. Scoping of a higher-tiered ERA may be necessary for sites requiring implementation of remedial action for a large areal extent and/or multiple years of remediation, and sites with complex ecosystems or trophic levels.

The two prime objectives of this type of ERA are: 1) the development of remediation goals to be applied to site cleanup, and 2) development of comparative risk assessments between different remedial options.

RISK MANAGEMENT - FOCUS ON SITE CLOSEOUT

The National Academy of Sciences (NAS) defines risk management as "a process of weighing policy alternatives and selecting the most appropriate regulatory action, integrating the results of risk assessment with engineering data and with social, economic and political concerns to reach a decision" (NRC 1983).

NAS has identified four key components for managing risk and resources: public participation, risk assessment, risk management, and public policy decision-makers (NRC 1994). Risk characterization is considered the "bridge" or "interface" between risk assessment and risk management. EPA recommends that risk characterization should be clearly presented and separated from any risk management considerations. EPA (1995d) policy indicates that risk management options should be developed using risk input and should be based on consideration of all relevant factors, both scientific and non-scientific.

Consistent with NAS, USACE has developed the HTRW risk management decision-making (RMDM) process. This process identifies factors to consider when making decisions, developing and recommending options, and documenting of risk management decisions.

The process establishes a framework to manage risk on a site-specific basis. It emphasizes that risk management must consider the strengths, limitations and uncertainties inherent in the risk assessment; the importance of public and other stakeholders' input; and other non-risk factors.

Risk and uncertainty are important factors to be considered in RMDM (EPA 1991d, 1995d). Other factors, including the customer's and stakeholders' concerns, cost, schedule, value of resources to be protected, political, and technical feasibility are also to be considered before selecting the best option for a project decision. The consideration of risk is critical, since site actions are driven by statutes and regulations which explicitly require the "protection of human health and the environment". This requirement focuses on the acceptability of site risks from the potential actions.

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**ADDRESSING DATA GAPS AND UNCERTAINTY IN ECOLOGICAL
RISK ASSESSMENTS: CASE STUDIES IN THE ARMY**

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INTRODUCTION

Traditional desk-top model approaches to ecological risk assessments (ERAs) are helpful in the preliminary stages of characterizing risk (i.e., for screening). When used in this context, they often use default values for exposure characterization which tend to overestimate rather than underestimate risk (Wentzel et al. 1994). For this reason models are helpful at sites where many substances are involved, and where a focussed approach requires screening out substances which are unlikely to have an influence on the ecosystem. Models also give a relative value (i.e., Hazard Quotient) of suspected toxicity for each substance which help to rank the priority of these substances for any specific site. However, using such 'conservative assumptions' often provides the risk manager a distorted view of the actual toxic effects of these substances in the field. Using these modeled estimates as actual representations of risk are problematic, since exposure and toxicity criteria are often not specific for the site, and rarely are ecologically relevant criteria used. Further, a desk-top modeled approach rarely addresses indirect effects of toxicity (i.e., factors which describe inter- and intraspecific interactions; see USEPA 1993, DeAngelis 1994). These factors have the potential to greatly reduce or increase risk, and some in themselves have been identified as primary density regulating forces (e.g., predator avoidance, increasing searching time, altering mate-recognition factors, etc.; Marcot 1994). Therefore, the use of site-specific exposure criteria (e.g., model validation) and relevant toxicological criteria, combined with field observations are important to accurately characterize risk and are useful to the risk manager in determining which substances are most likely to adversely affect the ecosystem.

Specifically, traditional models used for ecological risk assessments are lacking in that they:

- Assume factors which affect individuals will have a proportional effect on populations;
- Assume chemicals in prey and in media are 100% available;
- Ignore synergistic or antagonistic properties of exposure and effect;
- Do not consider the effects of selection on populations where initial exposures have occurred in the past;
- Ignore any indirect ecological effects resulting from exposure, e.g., depleted food resources, enhanced interspecific competition, altered mate recognition behavior, decreased predator avoidance, altered patterns of habitat use, dispersal, immigration, etc.; and,
- Rely upon a very limited toxicological data base, where species-specific and populationally relevant (i.e., developmental, reproductive, and immunological) data are unavailable, resulting in gross uncertainty and data gaps where many compounds and species of concern are assessed.

Quantitatively addressing complex interactions such as these are difficult and often infeasible. Further, the information gaps in community- and population-specific ecology are too prevalent and the interactions too complex to be considered in a model. However, observing the "big picture" at any site can provide ecologically-relevant data which can help to refine the model and address uncertainty (ecological and toxicological) for substances which fail the screen.

This manuscript is designed to focus on these uncertainties, and present methods used by U.S. Army Center for Health Promotion and Preventive Medicine (USACHPPM) to refine models

and support conclusions drawn in ecological risk assessments.

Receptor Characterization:

Accurately describing community structure in and around areas of concern is vital in the problem formulation and risk characterization phases. Proper site-specific receptor selection cannot be made without first determining which species compose or are likely to disperse into areas of concern. To accomplish this it is necessary to consider “all” factors which may influence community composition and species abundance. This often requires a seasonal approach to sampling whereby seasonal fluctuations in density, life history, and area use are considered. Extensive time and effort may not always be required. Local natural heritage organizations, ornithological societies, and natural history societies can often provide site-specific information which can help in designing methods to maximize the probability of detection. Moreover, integration of other sampling methods with receptor characterization methods (e.g., exposure characterization methods) can help provide additional information and reduce effort.

Complete receptor identification aids in describing the diversity in the community. Biodiversity of the site can help in addressing overall health of the system in addition to becoming a tool in addressing public concerns.

A multi-faceted approach to receptor identification can provide the greatest probability of achieving accurate data. Choosing the correct methods can provide additional information which may help to address other questions or gaps. Constrained modeled approaches may only address

toxicity criteria resulting from direct exposure, and not account for other important xenobiotic-caused influences (i.e., indirect effects, reproductive activity, toxicity data gaps, etc.). With terrestrial vertebrates, combining point counts with capture methods can provide this information. For birds, including capture-mark-recapture techniques (i.e., banding) provides information on species which are secretive, including reproductive activity, fat accumulation (indicator of food abundance), and age (often correlated to reproductive success; Saether 1990, Johnson *in Prep.*). Recapture need not be necessary but can provide additional evidence for reproductive residence at the site. Often birds engaged in the feeding of nestlings have a reduced home range and present a sensitive life stage for understanding the acute effects of some compounds.

An opportunity to test alternative methods became available at several Army sites. One was J-Field, at Aberdeen Proving Ground, Maryland. J-Field is a multi-purpose site used as a burning ground, demolition, and disposal area for white phosphorous, riot control agents, and high explosives. Previous investigations by Nemeth (1989) found that surface soil residues were of predominantly metals and organochlorines. The methods presented here were designed to provide supplemental information for model refinement and to address toxicological data gaps in the ecological risk assessment.

Qualitative point counts for birds were combined with mist netting techniques at J-Field. Point counts were conducted at and near the site, during each season in the course of a year. Mist netting activities occurred twice weekly for two months during the spring to assess breeding

activity and to consider exposures to migrants. Over 102 species were observed, with the Bald Eagle (*Haliaeetus leucocephalus*) and the Least Tern (*Sterna antillarum*) identified as species of special concern.

Amphibian species identification included qualitative observations (aural and visual observations) as well as species counts of each previously identified habitat of concern (see exposure characterization). All reptiles encountered during site visits were recorded including location and date. A total of 10 species of amphibians and 10 species of reptiles were observed at J-Field.

When developing data quality objectives, the selection of an appropriate site-relevant species are also important for model refinement. The use of a "generic small mammal" for the sake of model development often increases uncertainty. In another case-specific instance, our model validation efforts drastically lowered (by an order of magnitude) a contractor's desk-top model predictions of unacceptable risk to three receptors at each of seven distinct areas of concern. Substitutions to the species to be modeled were used in two different ways. First, a generic "small mammal" was replaced with two species (deer mouse, prairie vole) with a validated site presence, and for which there is much available bioenergetics information (such as metabolic rate, normalized ingestion rate, dietary composition, assimilation efficiencies). Secondly, two avian species used previously in the model were replaced with two others which were more site-specific and thus more relevant. One of the two species (Upland Sandpiper: *Bartramia longicauda*) has a rather specific habitat requirement, being a nearly solitary inhabitant of tall

grasslands. The other was the Northern Bobwhite (*Colinus virginianus*), where knowledge of dietary composition is fairly complete and there are adequate toxicity data. Table 1 provides a partial list of influential criteria for food ingestion pathway evaluation. This table compares how these criteria were treated in a desktop modeling exercise and USACHPPM's validation of that effort.

Exposure Characterization:

Conservative assumptions used in screening approaches and as defaults where species-specific criteria do not exist often translate into an inaccurate perception of risk. The default values used, e.g., 1 for bioaccumulation (i.e., biological transfer factors; BTFs), are prime culprits in overestimating exposure. These defaults assume 100% of the chemical concentration in soil is transferred to biota. The evidence presented here suggests that this is not true; that many substances detected in soil may not be bioavailable (see Hrudey et al. 1996 for a review). Since ingestion often is considered as the primary exposure route, refining ingested dose estimates are important. This involves chemical analysis of body-burden concentrations of the primary food source for the receptor of concern (used for model validation), and compares this concentration to that of the media (e.g., surface soil) to calculate a BTF. This BTF was also used as a value to be used directly in a food web model.

At another site, we refined the BTF component of the food ingestion pathway analysis with the collection of site biota. Insects and vegetation were collected and co-located at the centers of each of the seven areas of concern were sampled to determine the actual contaminant

concentration (principally metals) in the prey species of the receptors. Three different collection techniques were used for insects (sweep net, pitfall trap, bait trap). The assumed soil- and vegetation-borne metals-to-insects BTF of 1.0 was found to be true in only three instances ($N = 21$, 14%) throughout the seven locations. Those instances where the BTF was at 1.0 or greater were at those locations where there were lower chemical concentrations (i.e., concentrations similar to site background). In most instances, a clear trend of decreasing bioaccumulation with the increasing soil metals concentrations was apparent (Figs. 1&2). These data suggests the inability of an insect to accumulate no more than some maximum amount, irrespective of media concentration. Mechanisms for this observation have been proposed by others. Miura and Clarkson (1993), investigating specific mammalian cell lines, found evidence suggesting a glutathione-related efflux system. Other investigators have found in bacteria, metal-specific, protein complexes in the cell membrane which were responsible for metal ion efflux (Brown et. al. 1992, Kaur and Rosen 1992). It is therefore reasonable to suggest that these characteristics could be selected for in insect populations. Hence, especially where the concentrations were highest, limited uptake was an anticipated finding.

It is important to note that the biotransfer factors decreased with increasing soil concentration, often by an order of magnitude (e.g., 0.5 to 0.07). The actual quantities of metals taken up from the soil may have been greater where the biotransfer factors were lowest, rather than where they were they highest.

When completing insect characterization, prime considerations must be given to the applicability

of the food source to the consumer, life stage of the food source (e.g., larval vs. migratory adult, fruiting period, etc.), and ecology of the consumer and source. The applicability of whole-body burden analysis for migratory reproductive stages of lepidoptera collected at the site of concern may show no correlation to media results; i.e., collection and chemical analysis of migratory or highly vagile organisms are inappropriate. Further, collecting relatively sessile chitinous adult insects may not be a relevant food source to an invertivorous bird such as an American Woodcock. These issues must be considered when planning a strategy.

Chemicals of concern at J-Field included several organochlorines and metals (Nemeth 1989). Small mammals were trapped adjacent to areas of concern and amphibians were trapped at specified habitats. These habitats were predominantly craters formed from explosive ordinance which were filled with water and thus conducive for amphibian use. Since some of these habitats were either created from high explosive detonation or receive surface run-off from areas where munitions are detonated, the tissue was analyzed for munition and munition breakdown products as well as for selected metals and organochlorines.

Chemical analysis of small mammals and amphibians at J-Field yielded traces of DDE (p,p' dichlorodiphenyl dichloroethylene) and PCBs (polychlorinated biphenyls, e.g., Aroclor 1260). Concentrations of barium, chromium, and lead were found in frogs (*Rana utricularia*); traces of DDE and PCBs were found in small mammals (*Peromyscus leucopus*). A single frog from Crater 5 had traces of TNT (2,4,6-trinitrotoluene) and metabolites (Johnson et al. 1995). These data were all lower than modeled estimates for these receptors, were used to derive site-specific

BTFs and to refine the food web model for J-Field. Although potentially informative, no analyses were performed on amphibians from any reference locations nor were any collected.

Reproductive Performance:

Describing the relative quality of a habitat for any species is often assessed through measures of fecundity. This requires measuring the reproductive performance of the species of concern.

Stresses, due to direct (affecting reproduction) or indirect chemical contamination (e.g., changes in food abundance, predator densities, phytotoxic effects which alter forest physiognomy, etc.) will be ultimately realized by reduced offspring production. Further, by measuring reproductive performance, we are measuring the net effect of exposure; including possible synergism and intensity of exposure to multiple contaminants and relating it to the core of ecological risk: population viability. Abundance estimates (when not supported by other data), are only useful in the identification of possible receptors, and may not accurately reflect contaminant-related stresses or reduced reproductive performance in populations consisting of primarily immigrants (i.e., sink habitats; Van Horne 1983). Measures of reproductive performance can be compared to literature or control results to help support model conclusions of risk.

Reproductive performance of spotted salamanders (*Ambystoma maculatum*) and Eastern Bluebirds (*Sialia sialis*) were measured at J-Field. Salamander reproductive performance was measured at associated craters formed from explosive detonation and subsequently accumulating water. Salamander reproductive performance was determined through measures of pooled egg mass weight associated with each crater. The craters were of similar surface area and found not

to be significantly different in size (χ^2 goodness of fit: $N = 5, 15; df = 1, p = 0.19$). Differences in seasonal rainfall amounts and variations in crater recharge made accurate volumetric measurements impossible, though depth was not suspected to be significantly different. Pooled egg mass weight was then correlated with sediment metal concentrations. Sediment data were used and considered most relevant given the breeding and life history of the subject.

Additionally, soil chemical concentrations surrounding these sites were lower than sediment chemical of concern (COC) concentrations, and therefore were considered conservative. Surface water data were unavailable for each site, though concentrations of metals in surface water were lower than the sediment concentrations for those sites where there were data. The slopes were not significantly different than zero for each compound tested (Table 2), which indicated no obvious effect of metal concentration in the sediment could be correlated to the mass of eggs laid. Further, larval hatch was qualitatively observed either by removing a section of eggs in native water and returning them to the lab for subsequent observations or returning to the site for evidence of live larvae. All sites hatched viable larvae.

Reproductive performance of a representative avian receptor, Eastern Bluebirds, was also measured at J-Field. Bluebird boxes ($N = 22$) were placed in and surrounding each area of concern; more than could be used based on area and habitat availability (Johnson et al. 1995). Boxes were placed in early March, and monitored for activity at least monthly. When there was evidence of nesting activity, boxes were checked and nestlings banded prior to fledging. Results were calculated on an annual fledgling production/female basis. A control site on similar habitat characteristics was established 21 miles north of J-Field. Here bluebird production was less than

at J-Field, and was attributed primarily to House Sparrow (*Passer domesticus*) predation and increased human activity. J-Field bluebird production values were similar to literature values (J-Field, N = 3; Pitts 1988, N = 36), indicating that there were no profound toxic effects which would be realized on a populational level (Fig. 3). Tree Swallows which nested at J-Field were also productive, habituating boxes not used by bluebirds (N = 2). Clutch sizes were consistent with published data (not presented).

DISCUSSION

These cases provide examples of alternative methods and solutions for addressing uncertainty and data gaps inherent in modeled approaches to ecological risk assessment. Supplementing model results with validation efforts are important when addressing the question of ecological risk, and may result in cost-effective outcomes considering the current conservative model defaults used and the remediation costs associated with them. Further, it adds to our understanding of the fate and transport of xenobiotics in communities and ecosystems.

Questions of bioavailability, background concentrations, combined effects of many compounds in biological systems and the extrapolation of toxicity data to relevant site-specific receptors are important considerations when attempting to estimate ecological effects. Any one of these could greatly contribute to risk; the challenge is to determine which are actual and which are not. The collection of additional site-specific biological data is important in determining which are *most likely* to be the dominant risk contributors. This report provides information which suggests that default values used in determining BTFs and other exposure criteria may significantly

overestimate exposure.

The collection and analysis of measures of reproductive performance are valuable in assessing ecological risk from xenobiotics since they are a result of the net effect of organism health and thus habitat quality on a populational level. However, annual fluctuations in reproductive output caused by other environmental influences may be difficult to discriminate from those resulting from xenobiotic exposure (Brewer and Hummel 1994, Marcot 1994). To ameliorate these fluctuations, large sample sizes and long term monitoring are often required. Many sites limited in size may not sufficiently address these criticisms. Regardless, these data can be valuable for identifying catastrophic xenobiotic effects, particularly where evidence of reproductive immigrants (i.e., naive individuals) can be found and quantified. However, naive animals introduce the uncertainty of past exposures, and further, may not exhibit toxicity associated with compounds which slowly bioaccumulate as would be the case for native individuals.

Comparing offspring production with appropriate literature values (often where large samples are used) can provide a range of expected reproductive outcomes, which can be used to make qualitative judgments, although continued populational sustainability cannot reliably be addressed. Choosing methods such as these which analyze community or population health are excellent holistic techniques that help evaluate these criteria.

Care must be used in applying these methods and interpreting these results. Small sample sizes constrained by isolated habitat types and surveys which only include a single year of

observations may produce variable data, given the natural fluctuations common in many populations. However, population level impacts resulting from xenobiotic exposures should not be slight, but dramatic changes which should be extreme compared to natural fluctuations in reproductive performance often observed. Further, by inspecting offspring production through its developmental progression may help to identify the toxic mechanisms (e.g., organochlorine exposure in birds); often the most sensitive life stage where the toxicity of many compounds can be most influential.

It is logical that relatively minor xenobiotic influences (e.g., depressed immunofunction) and other natural and/or anthropogenic disturbances could combine to significantly impact a population. These assays assume that these events are rare, and that if true, dispersal into these areas during periods of low stress will likely result in limited changes in density. The methods presented here were designed to provide pieces to the puzzle of addressing ecological risk; to be useful in describing effects which occur on an ecological level of organization, and not only at an individual level. These types of assays address these concerns which are currently lacking in many ecological risk assessments.

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Table 1. Comparison of approaches used in incorporating food ingestion pathway components in an ERA.

Factor	Modeled ERA	Modeled Validation Effort (USACHPPM)
Receptor species selection	Generic	Specific; bioenergetics understood
Contaminant uptake by prey from media	Model from soil concentration + assume biotransfer factor is 1.0	Collect prey and measure concentrations to determine actual dose
RISK =	Unacceptable risk to all receptors at all locations	Marginal risk to one receptor at one location

Table 2. Regression analyses of sediment metal concentrations to pooled egg mass weight.

Independent Variable	Slope	p	r ²	$\beta - 1^*$
Aluminum	0.10	0.23	0.59	0.17
Arsenic	331.6	0.48	0.54	<0.0001
Barium	-8.41	0.82	0.03	0.04
Beryllium	2239.1	0.32	0.47	0.13
Calcium	-2.22	0.64	0.13	0.64
Cadmium	603.2	0.48	0.53	<0.0001
Chromium	65.1	0.47	0.28	0.08
Cobalt	-917.7	0.41	0.65	<0.0001
Copper	-4.43	0.70	0.09	0.05
Iron	0.03	0.09	0.82	0.32
Potassium	0.94	0.76	0.06	0.04
Magnesium	-0.55	0.71	0.08	0.05
Manganese	-17.4	0.60	0.16	0.06
Sodium	-11.6	0.47	0.29	0.09
Nickel	-554.6	0.22	0.88	<0.0001
Lead	-25.2	0.46	0.29	0.09
Vanadium	48.3	0.33	0.45	0.13
Zinc	-6.19	0.45	0.30	0.09

* $\beta - 1$ was determined at $\alpha = 0.05$

Fig. 1. Soil concentration relative to biotransfer factor (BTF) of sampled insects for copper.

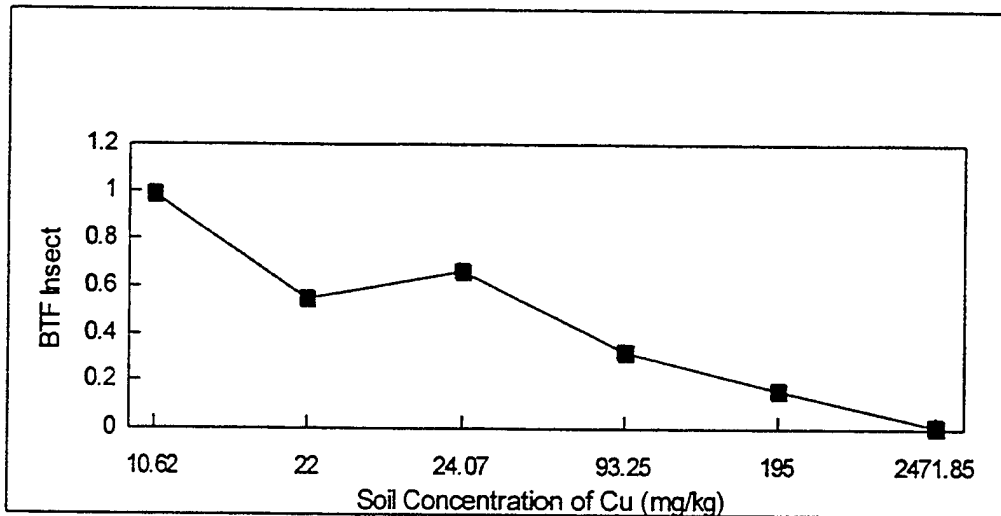


Fig. 2 Soil concentration compared to biotransfer factor (BTF) of sampled insects for lead.

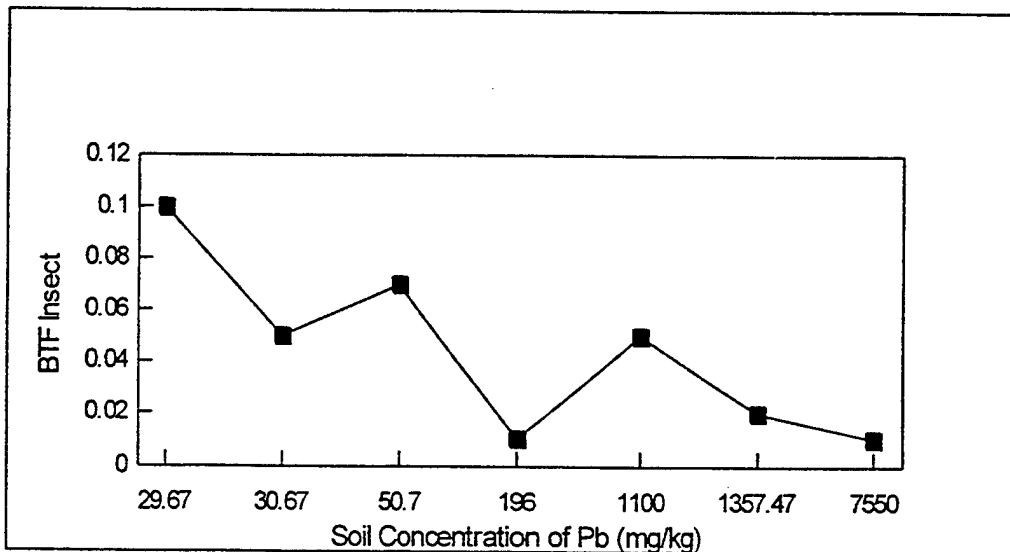
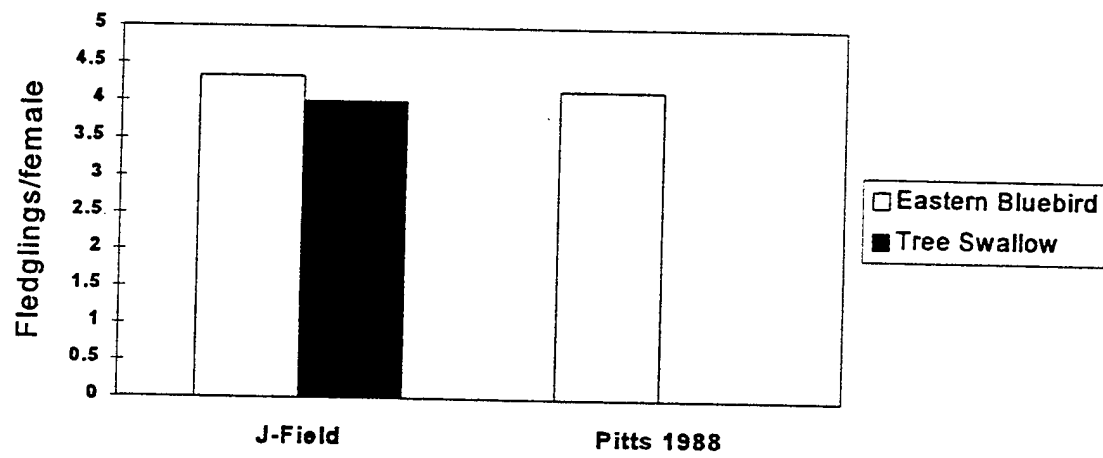


Fig. 3. Reproductive performance of Eastern Bluebirds at J-Field, APG (Johnson et al. 1995).



**ABSTRACT OF PRESENTATION:
ECOLOGICAL RISK ASSESSMENT
APPLICATIONS IN THE U.S. AIR FORCE**

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The U.S. Air Force installations occupy approximately 9,000,000 acres of land in the United States and its territories. About 12% of the land is used for runways, hangers, and various mission-related activities. The remainder is needed to ensure the safety of flying operations. The remaining acreage includes wetlands, threatened and endangered species, as well as many unique biological and geophysical resources. Most ecological risk assessments at Air Force bases are performed through contract mechanisms that address Clean Water Act, Clean Air Act, and RCRA hazard identification and CERCLA and RCRA cleanups. Contractors generally follow EPA's Framework for Ecological Risk Assessment. Problems arise when many interested parties disagree on how to do an ecological risk assessment and what the results imply in terms of making remedial decisions.

**ABSTRACT OF PRESENTATION:
A PERSPECTIVE ON ECOLOGICAL RISK
ASSESSMENT AT EPA**

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The U.S. Environmental Protection Agency (EPA) is placing increasing emphasis on using risk-based approaches as an integral part of the decisionmaking process. Agency-wide guidelines for ecological risk assessment proposed by EPA's Risk Assessment Forum are being developed to address the diversity of ecological risk approaches used in Agency programs. This paper will highlight elements of the proposed ecological risk assessment guideline and discuss significant issues encountered during its development.

**ABSTRACT OF PRESENTATION:
USING ECOLOGICAL RISK
ASSESSMENT AS A TOOL IN
ENVIRONMENTAL DECISIONMAKING: A
CASE STUDY OF THE NATIONAL GYPSY
MOTH MANAGEMENT PROGRAM**

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The National Environmental Policy Act (NEPA) requires federal agencies to consider the environmental consequences of their actions in the decision making process. Ecological risk assessments can provide a mechanism for comparing the risks of alternatives considered in NEPA documents (e.g., environmental impact statements, environmental assessments).

The U.S. Forest Service and USDA Animal and Plant Health Inspection Service (APHIS) have prepared a programmatic environmental impact statement (PEIS) to assess the impacts of the National Gypsy Moth program. The National Gypsy Moth PEIS compared five alternatives including a number of different control techniques, and a sixth alternative involving no action. The cause of ecological stress differs between the no action alternative which considers the impacts of uncontrolled gypsy moths and the other five treatment alternatives which consider impacts of applying diflubenzuron, *Bacillus thuringiensis*, nucleopolyhedrosis virus, and gypsy moth pheromone.

This paper describes the methodology used to estimate and compare the risks associated with various control tactics (diflubenzuron, *Bacillus thuringiensis*, nucleopolyhedrosis virus, and gypsy moth pheromone) and no action (increased gypsy moth populations). The program area was divided into two ecosystems, the urban/suburban and the undeveloped forest ecosystems, based on the fate and transport of the insecticides in the

environment. Five ecological endpoints were selected (nontarget species, forest condition, water quality, microclimate, and soil productivity and fertility). A probabilistic quantitative assessment of risk to nontarget species was performed using Monte Carlo simulation to relate exposure to toxicity. Qualitative analysis of risk was performed for the other endpoints.

Ecological risk assessments are not routinely performed to support NEPA documents, although this type of analysis provides the detailed ecological information that is very useful in the environmental consequences section of a PEIS. The methodology used in ecological risk assessment at the broad programmatic scale of analysis differs from techniques used in site-specific remediation studies. The Ecological Risk Assessment for the National Gypsy Moth Management Program is an illustration of how ecological risk assessments can be tailored to address specific management questions.

SESSION III
BIOLOGICALLY BASED MODELING APPLICATIONS IN
RISK ASSESSMENT OF TOXIC SUBSTANCES

**INVESTIGATION OF THE POTENTIAL IMPACT OF BENCHMARK DOSE
AND PHARMACOKINETIC MODELING IN NONCANCER RISK
ASSESSMENT**

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ABSTRACT

There has been relatively little attention given to incorporating knowledge of mode of action or of dosimetry of active toxic chemical to target tissue sites in the calculation of noncancer exposure guidelines. One exception is the focus in the revised Reference Concentration (RfC) process on delivered dose adjustments for inhaled materials. The studies reported here attempt to continue in the spirit of the new RfC guidelines by incorporating both mechanistic and delivered dose information using a physiologically-based pharmacokinetic (PBPK) model, along with quantitative dose-response information using the benchmark dose (BMD) method, into the noncancer risk assessment paradigm. Two examples of the use of PBPK and BMD techniques in noncancer risk assessment are described: methylene chloride, and trichloroethylene. Minimal Risk Levels (MRLs) based on PBPK analysis of these chemicals were generally similar to those based on the traditional process, but individual MRLs ranged from roughly 10-fold higher to more than 10-fold lower than existing MRLs which were not based on PBPK modeling. Only two MRLs were based on critical studies which presented adequate data for BMD modeling, and in these two cases the BMD models were unable to provide an acceptable fit to the overall dose-response of the data, even using pharmacokinetic dose metrics. A review of ten additional chemicals indicated that data reporting in the toxicological literature is often inadequate to support BMD modeling. Three general observations regarding the use of PBPK and BMD modeling in noncancer risk assessment were noted. First, a full PBPK model may not be necessary to support a more accurate risk assessment; often only a simple pharmacokinetic description, or an understanding of basic pharmacokinetic principles, is needed. Second, pharmacokinetic and mode of action considerations are a crucial factor in conducting noncancer risk assessments which involve animal-to-human extrapolation. Third, to support the application of BMD modeling in noncancer risk assessment, reporting of toxicity results in the toxicological literature should include both means and standard deviations for each dose group in the case of quantitative endpoints, such as relative organ weights or testing scores, and should report the number of animals affected in the case of qualitative endpoints.

INTRODUCTION

The purpose of this study was to investigate the potential impact of physiologically-based pharmacokinetic (PBPK) modeling and benchmark dose (BMD) modeling in the derivation of Minimal Risk Levels (MRLs). The first phase of the study focused on two chemicals of interest to ATSDR for which PBPK models were known to be available: methylene chloride (MC) and trichloroethylene (TCE). For each MRL currently defined for these two chemicals, the study which had served as the basis for the current MRL was evaluated from the standpoint of its suitability for BMD modeling. Each MRL was then re-calculated using PBPK modeling and, if appropriate, BMD modeling.

In the second phase of the study, a preliminary evaluation of ten additional chemicals of interest to ATSDR was performed to determine their suitability for BMD modeling as well as the availability and potential impact of pharmacokinetic models. The purposes of this phase of the study were (1) to evaluate the generality of the potential application of PBPK and BMD techniques for the development of MRLs, and (2) to identify those chemicals which would be the best candidates for further study.

Physiologically Based Pharmacokinetic Modeling

One of the scientific areas for which there has been considerable interest regarding potential applications in risk assessment is physiologically-based pharmacokinetic (PBPK) modeling. Advantages of applying PBPK modeling in risk assessment have been discussed both for cancer (Clewell and Andersen, 1985; 1989; NRC, 1987; USEPA, 1989; Frederick, 1993) and noncancer endpoints (Reitz *et al.*, 1988; Beck *et al.*, 1993; Clewell and Jarnot, 1994). In addition, the use of PBPK modeling has been recommended to improve route-to-route extrapolation (Gerrity and Henry, 1990) and the estimation of risk for chemical mixtures (Mumtaz *et al.*, 1993). Briefly, PBPK modeling attempts to describe the relationship between external measures of applied dose (e.g., amount administered or concentration in food, water, or air) and internal measures of delivered dose (e.g., amount metabolized or concentration in the tissue displaying the toxic response), using as realistic a description of mammalian physiology and biochemistry as is necessary and feasible. A number of excellent reviews have been written on the subject of PBPK modeling (Himmelstein and Lutz, 1979; Gerlowski and Jain, 1983; D'Souza and Boxenbaum, 1988; Leung, 1991).

In the past, the guiding paradigm for non-cancer risk assessments has been largely empirical. MRL calculations have usually relied on direct toxicity observations derived from studies conducted with specific routes of administration. No-observed-adverse-effect-level (NOAEL) or lowest-observed-adverse-effect-level (LOAEL) estimates derived from the data have then been adjusted by the application of uncertainty factors to estimate reference concentrations or reference doses. There has been relatively little attention given to incorporating knowledge of mode of action or of dosimetry of active toxic chemical to target sites in these calculations. In addition, the default uncertainty factors also fail to provide a role for mechanistic information or pharmacokinetic data in standard setting for these various endpoints. One exception is the focus in the revised RfC process on delivered dose adjustments for inhaled materials (USEPA, 1994). The study reported here attempts to continue in the spirit of these new guidelines by incorporating delivered dose information predicted by a PBPK model into the risk assessment paradigm for MRLs.

Benchmark Dose Modeling

When possible, dose-response analysis of the critical studies underlying the MRLs was also performed using the Benchmark Dose (BMD) methodology (Crump 1984, 1995). When used with exposure concentrations, this approach is sometimes referred to as the Benchmark Concentration (BMC) methodology; however, the abbreviation BMD will generally be used for both routes of exposure in this document. The BMD (BMC) is the dose (concentration) predicted to result in a specified amount of increased risk (referred to as the "benchmark risk"). The BMD or BMC is calculated using a statistical dose-response model applied to either experimental toxicological or epidemiological data. A statistical lower bound on the BMD or BMC (referred to as the BMDL or BMCL, respectively) has been proposed as a replacement for the traditional NOAEL, which must be selected from one of the actual experimental dosing levels, in the setting of acceptable exposure limits (USEPA 1994, Gaylor and Slikker, 1990).

In the traditional approach for estimating a NOAEL from animal data, the response at each of the experimental doses is compared statistically with that in the controls, and the NOAEL is defined as the lowest dose showing no statistical difference. The benchmark approach has several advantages over the traditional NOAEL approach: (1) the benchmark approach makes better use of the dose-response information inherent in the data, (2) the benchmark approach appropriately reflects the sample size of a study (Smaller studies tend to result in smaller BMDs, whereas the opposite is true

for traditionally derived NOAELs), (3) the benchmark approach does not require arbitrary categorization of the data in epidemiological studies, (4) the benchmark approach does not involve difficult and argumentative "all or nothing" decisions, such as determining whether or not a NOAEL was observed in a particular experimental dose or exposure category, and (5) a benchmark estimate of the NOAEL can be determined even when effects are observed in the lowest experimental dose group or exposure category. In its "Interim Methods for Development of Inhalation Reference Concentrations" (USEPA, 1994), the EPA stated "This novel method utilizes more of the available data than the current methodology ... It also addresses to some degree several of the criticisms of the current approach, such as the use of dose-response slopes and the number of animals tested in defining NOELs."

A study recently conducted for the EPA (Allen *et al.*, 1994) compared BMDs with traditionally-derived NOAELs for 424 sets of quantal animal data. The results of this study showed that lower bound BMDs based on an additional risk of 10% ($BMDL_{0.1}$) were smaller than the corresponding traditional NOAEL for more than 75% of the data sets and were less than the traditional NOAEL by an average factor of 2.9; lower bound BMDs based on an additional risk of 5% ($BMDL_{0.05}$) were smaller than the corresponding NOAEL for between 90% and 95% of the data sets and were less than the NOAEL by an average factor of 5.9; lower bound BMDs based on an additional risk of 1% ($BMDL_{0.01}$) were smaller than the corresponding NOAEL for more than 95% of the data sets and were less than the NOAEL by an average factor of 29. Based on this analysis, use of 10% additional risk appears to provide a BMDL that corresponds more closely to a traditional NOAEL than use of 5% or 1%. BMDLs corresponding to an additional risk of 10% also have the advantage that they are likely to be less dependent upon the dose-response model than BMDLs corresponding to additional risk of 1% or 5% (Crump, 1984). These analyses suggest that use of a lower bound for 10% additional risk would increase the conservatism in the determination of RfCs and RfDs by a factor of about 2 to 3 (i.e., would decrease RfCs and RfDs by a factor of 2 to 3 on average) as compared to the traditional NOAEL approach. In this analysis, benchmark risks (BR) of 10%, 5% and 1% were calculated to evaluate the sensitivity of the predicted NOAEL to the selection of BR, but only the result for 10% increased risk (the $BMDL_{0.1}$) was used as an estimate of the NOAEL.

EVALUATION OF MRLs FOR METHYLENE CHLORIDE AND TRICHLOROETHYLENE

In the following section, each of the current MRLs for MC and TCE are considered in turn. In each case, the critical study chosen by ATSDR as the basis of the MRL is summarized, and its suitability for BMD modeling is evaluated. Each MRL is then re-calculated using PBPK modeling and, if appropriate, BMD modeling.

Since the purpose of this effort was specifically to evaluate the potential impact of PBPK and BMD modeling techniques on the MRL process, all other aspects of the MRL process were kept the same to the extent possible. In each case, only the study chosen by ATSDR as the basis for the current MRL was evaluated; no attempt was made to consider alternative studies which might provide better data for PBPK or BMD modeling. The same uncertainty factors as in the current MRL were also used, except that when appropriate the animal to human uncertainty factor was reduced from 10 to 3 to reflect the consideration of pharmacokinetics, in accordance with ATSDR guidelines. In cases where dosimetric adjustments were made in the current MRLs the pharmacokinetic model was applied in such a manner as to perform an equivalent adjustment. In summary, any differences between the current MRLs and those calculated with the PBPK or BMD models solely reflects the impact of applying these techniques, and is not due to variations in other aspects of the MRL derivation process.

Methylene Chloride

The PBPK model for MC used in this study has been described previously (Andersen et al., 1987; Clewell, 1995a). The pharmacokinetic dose metrics available in the model include peak blood concentration of MC (CV_{max}), area under the concentration curve for MC in the blood (AUCB), and total metabolism per gram of liver (TMetL). Three MRLs are currently provided for MC (ATSDR, 1993); they will each be discussed in turn.

Acute Inhalation:

Critical Study:	Winneke (1974)
Subjects:	Female humans
Exposures:	0, 300, 500, 800 ppm; 3-4 hours

Effect:	Behavioral (decreased performance)
Measure:	Performance tests
Type of data:	Continuous, grouped
Data presented:	Number of subjects, mean response, and exposure concentration for each group.
Suitability for BMD:	Inadequate reporting of data (no standard deviation data)
NOAEL:	None identified
LOAEL:	300 ppm
Adjustment:	Daily time-weighted average
Uncertainty Factor:	100 (10 use of LOAEL, 10 variability)
Current MRL:	0.4 ppm

Benchmark dose modeling could not be performed on this study due to inadequate reporting of the data. For continuous data such as the test scores reported in this study, either the individual scores must be reported or the means and standard deviations for each group. Only the means were reported in this study.

The acute CNS effects of solvents such as MC display both a rapid onset and a rapid reversibility, and are likely to represent a direct physico-chemical effect of the parent chemical in the CNS tissue. A reasonable dose metric for such effects is the peak concentration of the parent chemical in the blood (assuming the CNS tissue/blood partition is roughly constant across species). For an inhalation exposure to an lipophilic, volatile chemical such as MC, the peak concentration (CV_{max}) is reached when the chemical achieves steady-state partitioning between air and blood. An alternative, and typically more conservative, dose metric is the area under the concentration curve (AUC) of the parent chemical in the blood (AUCB); this metric corresponds most closely to the default approach for calculating an MRL, in which the concentration is adjusted by the ratio of the actual duration of exposure to a continuous exposure.

Several steps are necessary to obtain an alternative MRL using the PBPK model. First, the model is run with human parameters for the exposure conditions of the LOAEL (300 ppm, 4 hrs). The resulting dose metrics are then divided by the uncertainty factor of 100 to obtain the target dose metrics for the MRL. The model is then run at various continuous exposure concentrations until the target dose metric is obtained. In this case, the target peak concentration was achieved at a

concentration of 6 ppm, while the target AUCB was obtained at a concentration of 0.8 ppm. Therefore the PBPK-based MRL for acute inhalation of MC is either 6.0 or 0.8 ppm, depending on whether the appropriate dose metric is the peak concentration or the area under the curve (AUC), respectively. As mentioned above, the use of the AUC is equivalent to the use of the time-weighted average concentration (that is, adjusting to equivalent continuous exposure) which is the approach typically used by ATSDR, and is the more conservative choice. Using this metric, the impact of applying PBPK modeling to the acute inhalation MRL for MC is to raise the MRL by a factor of 2.

Intermediate Inhalation:

Critical Study:	Haun et al. (1972)
Subjects:	Rats
Exposure levels:	0, 25, 100 ppm; 100 days, continuous
Effect:	Liver and kidney toxicity
Measure:	Pathological examination
Type of data:	Quantal, grouped
Data presented:	Number of subjects and exposure concentration for each group.
Suitability for BMD:	Inadequate reporting of data (no quantitative incidence data)
NOAEL:	None identified
LOAEL:	25 ppm
Adjustment:	None required
Uncertainty Factor:	1000 (10: use of LOAEL, 10: animal to human, 10: variability)
Current MRL:	0.03 ppm

Benchmark dose modeling could not be performed on this study due to inadequate reporting of the data. Although toxicity was reported, the number of animals affected at each dose was not.

In contrast to acute CNS effects, liver toxicity from solvents such as MC typically results from the generation of reactive species during the metabolism of the parent chemical. A reasonable dose metric for such effects is the total amount of metabolism divided by the volume of the liver (a pseudo-concentration of reactive metabolite).

Again, several steps necessary to obtain an alternative MRL using the PBPK model. First, the model is run for the exposure conditions of the LOAEL (25 ppm, continuous, in rats). The resulting dose metric is then divided by the uncertainty factor of 300 (instead of 1000, due to the discounting of the animal-to-human uncertainty factor from 10 to 3 for use of a pharmacokinetic model) to obtain the target dose metric for the MRL. The model is then run at various continuous exposure concentrations in the human until the target dose metric is obtained. In this case, the target total metabolism per gram of liver was achieved at a concentration of 0.2 ppm. Therefore the PBPK-based MRL for intermediate inhalation of MC is higher than the current MRL by a factor of about 7.

Chronic Oral:

Critical Study:	Serota et al. (1986)
Subjects:	Rats
Exposures:	0,6,55,131,249 mg/kg/d; 2 years
Effect:	Liver toxicity
Measure:	Pathological examination
Type of data:	Quantal, grouped
Data presented:	Number of subjects and exposure concentration for each group.
Suitability for BMD:	Inadequate reporting of data (no quantitative incidence data for non-neoplastic lesions)
NOAEL:	6 mg/kg/day
Adjustment:	None required
Uncertainty Factor:	100 (10: animal to human, 10: variability)
Current MRL:	0.06 mg/kg/d

Benchmark dose modeling could not be performed on this study due to inadequate reporting of the data. Although noncancer toxicity was reported, the number of animals affected at each dose was not.

As mentioned above, a reasonable dose metric for liver toxicity from MC is the total amount of metabolism divided by the volume of the liver. As before, the model is first run for the exposure conditions of the NOAEL (6 mg/kg/d MC in drinking water, in rats). The resulting dose metric is then divided by the uncertainty factor of 30 (instead of 100, due to the discounting of the animal-to-

human uncertainty factor from 10 to 3 for use of a pharmacokinetic model) to obtain the target dose metric for the MRL. The model is then run for humans at various doses of MC in drinking water until the target dose metric is obtained. In this case, the target total metabolism per gram of liver was achieved at a dose of 0.13 mg/kg/d. Therefore the PBPK-based MRL for chronic oral exposure to MC is higher than the current MRL by about a factor of 2.

Trichloroethylene

The PBPK model for TCE used in this study has also been described previously (Clewett et al., 1994, 1995a). The pharmacokinetic dose metrics available in the model include maximum blood concentration of TCE (CV_{max}), area under the concentration curve for TCE in the blood (AUCB), and area under the concentration curve for trichloroacetic acid (TCA) in the blood (AUCTCA). Four MRLs have been calculated for TCE (ATSDR, 1995); each will be discussed in turn.

Acute Inhalation:

Critical Study:	Stewart et al. (1970)
Subjects:	Humans
Exposures:	200 ppm; 5 days, 7 hr/d
Effect:	Behavioral (mental fatigue, sleepiness)
Measure:	Subject interview
Type of data:	Quantal, grouped
Data presented:	Number of subjects and exposure concentration for each group.
Suitability for BMD:	Inadequate reporting of data (no quantitative incidence data)
NOAEL:	None identified
LOAEL:	200 ppm
Adjustment:	Daily time-weighted average
Uncertainty Factor:	30 (3: use of minimal LOAEL, 10: variability)
Current MRL:	2 ppm

Benchmark dose modeling could not be performed on this study due to inadequate reporting of the data. Although toxicity was reported, the number of individuals affected was not.

As mentioned above for MC, a reasonable dose metric for acute CNS effects of solvents is the peak concentration of the parent chemical in the blood (CV_{max}), or alternatively, the AUC of the parent chemical in the blood (AUCB). First, the model is run for the exposure conditions of the LOAEL (200 ppm, 7 hrs/day in humans), and the resulting dose metrics are then divided by the uncertainty factor of 30 to obtain the target dose metrics for the MRL. The model is then run for humans at various continuous exposure concentrations until the target dose metric is obtained. In this case, the target peak concentration was achieved at a concentration of 8 ppm, while the target AUCB was obtained at a concentration of 2 ppm. Therefore the PBPK-based MRL for acute inhalation of TCE is either 8 or 2 ppm, depending on whether the appropriate dose metric is the peak concentration or the area under the curve (AUC), respectively. As mentioned for MC, the use of the AUC is equivalent to the use of the time-weighted average concentration (that is, adjusting to equivalent continuous exposure) which is the approach typically used by ATSDR.

Intermediate Inhalation:

Critical Study:	Arito et al. (1994)
Subjects:	Rats
Exposures:	0, 50, 100, 300 ppm; 6 weeks, 5 d/wk, 8 hr/d
Effect:	CNS effects (decreased wakefulness, reduced heart rate)
Measure:	Duration of wakefulness, heart rate
Type of data:	Continuous, grouped
Data presented:	Number of subjects, mean response, standard deviation, and exposure concentration for each group.
Suitability for BMD:	Adequate data are reported.
NOAEL:	None identified
LOAEL:	50 ppm
Adjustment:	Daily time-weighted average; ventilation to body-weight ratio
Uncertainty Factor:	300 (10: use of LOAEL, 3: animal to human, 10: variability)
Current MRL:	0.1 ppm

It should be noted that the dosimetric calculation in the current MRL does not appear to be appropriate for a chemical such as TCE. The dosimetric calculation used in the current MRL derivation is:

$$\text{HEC} = \text{TWA} \times (\text{QP animal} / \text{QP human}) \times (\text{BW human} / \text{BW animal})$$

where: HEC = human equivalent concentration

TWA = time-weighted average concentration in animal

QP = pulmonary ventilation

BW = body weight

This adjustment would be appropriate if TCE were a water-soluble gas that continues to accumulate in the blood throughout an exposure; that is, a Category 2 gas in the EPA RfC dosimetry scheme (USEPA, 1994). However, TCE should be treated as a relatively water-insoluble gas (EPA Category 3) that achieves a steady-state partitioning between the air and blood. The dosimetry calculation for Category 3 gases is:

$$\text{HEC} = \text{TWA} \times (\text{PB animal} / \text{PB human})$$

where: PB = the blood/air partition coefficient

and the PB ratio factor is only applied if it is less than one (USEPA, 1994). Using this approach, the MRL would be 0.04 ppm, rather than 0.1 ppm..

In the case of the acute inhalation MRL for TCE, the data reported in the critical study is adequate for Benchmark dose modeling. The models used for this continuous data were the polynomial model (THC) and Weibull (power) model (THWC). The results of the BMD modeling will be described after the discussion of the PBPK modeling results.

An appropriate dose metric in this case is once again the peak concentration or AUC of TCE in the blood. First, the model is run for the exposure conditions of the LOAEL (50 ppm, 8 hrs/day in rats). The resulting dose metrics are then divided by the uncertainty factor of 300 to obtain the target dose metrics for the MRL. The model is then run for humans at various continuous exposure concentrations until the target dose metric is obtained. In this case, the target peak concentration was achieved at a concentration of 0.25 ppm, while the target AUCB was obtained at a concentration of 0.1 ppm. Therefore the PBPK-based MRL for intermediate inhalation of TCE is either 0.25 or 0.1

ppm, depending on whether the appropriate dose metric is the peak concentration or the area under the curve (AUC), respectively.

The PBPK model was next used to obtain AUCB dose metrics for each of the doses used in the critical study. The BMD models were then run using either external concentrations or internal dose metrics (AUCB). The results for a range of benchmark risks are plotted in Figure 1. As discussed earlier, based on previous studies (Allen et al., 1994) the 95% lower bound on the dose associated with $BR = 0.1$ (the $BMDL_{0.1}$) is a reasonable estimate of the NOAEL. Using BMD modeling only (that is, modeling external concentration), the lowest $BMDL_{0.1}$ is for wakefulness, at 56 ppm. The fact that this concentration is actually above the concentration which was established to be a LOAEL by pairwise significance testing is certainly troubling; the reason for this discrepancy is the poor fit of the model to the data, as can be seen from inspection of Figure 1a.

In order to obtain a better fit of the dose-response in the lower concentration range, the models were rerun omitting the highest dose. The results are shown in Figure 2. The lowest $BMDL_{0.1}$ without the highest dose is for wakefulness at 31 ppm. As can be seen from inspection of Figure 2a, the models are now able to fit the dose response of the remaining data. Using this $BMDL_{0.1}$ as an estimate of the NOAEL, we can derive the MRL by adjusting to continuous exposure and dividing by an uncertainty factor of 30 (the same as for the current MRL, but eliminating the factor of 10 for use of a LOAEL). The resulting MRL is 0.25 ppm, a factor of 6 higher than the MRL of 0.04 based on the LOAEL. This result demonstrates the value of the BMD approach when dose-response data is available but a NOAEL has not been identified. Instead of applying the generic default of 10 for use of a LOAEL, the dose-response of the specific effect of concern can be used to determine where a NOAEL would have occurred. According to the BMD analysis of this dataset, a NOAEL would have been found less than a factor of 2 below the identified LOAEL.

The two techniques, PBPK modeling and BMD modeling, can also be used together. In some cases, such as when the dose-response of the data is nonlinear due to saturation of metabolism, the use of PBPK dose metrics rather than external doses can serve as a linearizing transformation, resulting in a better fit of the BMD model to the data (Clewett et al., 1995b). In this case, however, the use of the PBPK dose metrics did not appreciably change the BMD modeling results. As can be seen by comparison of Figures 1a and 1b, the use of the PBPK dose metric (AUCB) did not linearize the data; the nonlinearity in this case probably represents saturation of response rather than nonlinear

pharmacokinetics. Omitting the highest dose, the BMDL_{0.1} occurs at an AUCB of 8.8 mg-hrs/L. Dividing by the uncertainty factor of 30 produces the target dose metric of 0.3 mg-hrs/L, which was obtained with the PBPK model for continuous human exposure to 0.7 ppm. Thus the overall impact of BMD modeling (to estimate a NOAEL) and PBPK modeling (to correctly extrapolate across species) is to raise the MRL from 0.04 ppm (using the corrected default calculation) to 0.7 ppm, a factor of more than 17.

Acute Oral:

Critical Study:	Fredriksson et al. (1993)
Subjects:	Male mouse pups
Exposures:	0, 50, 290 mg/kg/d, days 10-16
Effect:	Developmental behavioral effects (decreased rearing onto hind legs)
Measure:	Observation
Type of data:	Continuous, grouped
Data presented:	Number of subjects, mean response, and dose for each group.
Suitability for BMD:	Inadequate reporting of data (no standard deviation data)
NOAEL:	None identified
LOAEL:	50 mg/kg/d
Adjustment:	None required
Uncertainty Factor:	100 (10: use of LOAEL, 10: animal to human)
Current MRL:	0.5 mg/kg/d

Benchmark dose modeling could not be performed on this study due to inadequate reporting of the data. For continuous data such as the data reported in this study, either the individual scores must be reported or the means and standard deviations for each group. Only the means were reported in this study.

It is not possible to unambiguously define the most appropriate dose metric for developmental effects from TCE. Likely candidates include the peak concentration and AUC for either TCE or one of its metabolites, such as TCA. The use of AUC is typically more conservative than peak concentration, and is less subject to uncertainty regarding the rate of oral absorption.

The steps necessary to obtain an alternative MRL using the PBPK model are somewhat more complicated than for the previous cases. The reason for the greater complexity is the long half-life of TCA in the body, which makes it necessary to model dosing over a prolonged period in order to estimate steady-state conditions. First, the model is run for the exposure conditions of the LOAEL: 50 mg/kg/d gavage for 7 days in the mouse pup (assumed to weigh 0.0035 kg, 10% of the adult body weight). The resulting dose metrics are then divided by the uncertainty factor of 30 (instead of 100, due to the discounting of the animal-to-human uncertainty factor from 10 to 3 for use of a pharmacokinetic model) to obtain the target dose metrics for the MRL. The model is then run for the human infant (with a weight of 7 kg, 10% of the adult body weight) at various doses in drinking water. In order to estimate the steady-state exposure, the model is run for two weeks and one week, and the dose metrics are subtracted to obtain an estimate of the 7-day dose metric. A comparison with the results of running the model for three weeks and two weeks and subtracting to estimate the 7-day dose metric (data not shown) produced an estimate which was only negligibly different. In this case, the target AUC for TCE was achieved at a dose of 0.015 mg/kg/d, while the target AUC for TCA was obtained at a dose of 0.05 mg/kg/d. Therefore the PBPK-based MRL for acute oral exposure to TCE is either 0.015 or 0.05 mg/kg/d, depending on whether the appropriate dose metric is the AUC of TCE or TCA, respectively. Thus the impact of applying PBPK modeling to the acute oral MRL for TCE is to lower the MRL by a factor of 10 to 33.

Intermediate Oral:

Critical Study:	Dawson et al. (1993)
Subjects:	Rat fetuses
Exposures:	0, 1.5, 1100 ppm in drinking water
Effect:	Cardiac congenital abnormalities
Measure:	Pathological examination
Type of data:	Quantal, grouped
Data presented:	Number of subjects, prevalence of abnormal hearts, and dosing for each group.
Suitability for BMD:	Adequate data reported
NOAEL:	None identified
LOAEL:	0.18 mg/kg/d
Adjustment:	None required

Uncertainty Factor: 100 (10: use of LOAEL, 10: animal to human)
Current MRL: 0.002 mg/kg/d

Although the data is technically adequate for BMD modeling, there is no apparent dose-response relationship for the effect; a change in dose of three orders of magnitude produces only a slight change in prevalence. Any quantitative use of this data should be considered suspect. Another significant concern for the proper interpretation of this study is the fact that the results are not reported by litter; therefore it is not possible to test for litter effects (that is, one or two litters could account for the bulk of the defective pups, producing a situation in which the number of affected pups was significant but the number of affected litters was not).

The models used for analysis of this quantal data were the polynomial model (THRESHLU) and Weibull model (THRESHW). The results of the BMD modeling will be described after the discussion of the PBPK modeling results.

The dose metrics used in this case are once again the AUC of TCE and TCA in the blood. First, the model is run for 7 days and 6 days, and the results subtracted to estimate a daily dose metric at steady-state for the exposure conditions of the LOAEL (0.18 mg/kg/d in drinking water in rats). The resulting dose metrics are then divided by the uncertainty factor of 30 (instead of 100, due to the discounting of the animal-to-human uncertainty factor from 10 to 3 for use of a pharmacokinetic model) to obtain the target dose metrics for the MRL. The model is then run in the same way for humans at various doses in drinking water until the target dose metric is obtained. In this case, the target AUCs were obtained at doses of 0.0004 and 0.0002 mg/kg/d in drinking water, for TCE and TCA respectively. Thus the PBPK-based MRL is a factor of 5 to 10 below the current value.

The PBPK model was next used to obtain AUC dose metrics for TCE and TCA at each of the doses used in the critical study. The BMD models were then run using either administered doses or internal dose metrics (AUCB and AUCTCA). The results for a range of benchmark risks are plotted in Figures 3 and 4. Using BMD modeling only (that is, modeling administered dose), the lowest BMDL_{0.1} is for dosing during pregnancy only, at 65 mg/kg/d. As with the previous application of BMD modeling, this dose is well above the concentration which was established to be a LOAEL by

pairwise significance testing, and the reason for this discrepancy is again the poor fit of the model to the data, as can be seen from inspection of Figures 3a and 4a.

As before, in order to obtain a better fit of the dose-response in the lower concentration range, the models were rerun omitting the highest dose. The results are shown in Figures 5 and 6. The lowest $BMDL_{0.1}$ without the highest dose is for dosing during pregnancy only, at 0.24 mg/kg/d. Not surprisingly, as can be seen from inspection of Figures 5a and 6a, the models are now able to fit the dose response of the remaining data (one dose and control). Using this $BMDL_{0.1}$ as an estimate of the NOAEL, we can derive the MRL by dividing by an uncertainty factor of 10 (the same as for the current MRL, but eliminating the factor of 10 for use of a LOAEL). The resulting MRL is 0.024 ppm, a factor of 12 higher than the MRL of 0.002 based on the LOAEL.

As can be seen by comparison of Figure 4a with Figures 4b and 4c, the use of the PBPK dose metrics (AUCB and AUC TCA) did not linearize the data; the nonlinearity in this case, if real, probably represents saturation of response rather than nonlinear pharmacokinetics. Omitting the highest dose, the $BMDL_{0.1}$ occurs at an AUCB of 0.0027 mg-hrs/L and an AUC TCA of 2.26 mg-hrs/L.. Dividing by the uncertainty factor of 3 produces target dose metrics of 0.001 and 0.75 mg-hrs/L, which are obtained with the PBPK model for continuous human exposure to 0.006 and 0.003 mg/kg/d, for AUC of TCE and TCA respectively. Thus the overall impact of BMD modeling (to estimate a NOAEL) and PBPK modeling (to correctly extrapolate across species) is to raise the MRL slightly.

Summary: Methylene Chloride and Trichloroethylene

PBPK modeling:

The impact of PBPK modeling on the MRLs for MC and TCE is summarized in Table 1. The impact is clearly chemical and exposure-route dependant. For MC, PBPK modeling produced higher MRLs for both inhalation and oral exposure, but particularly (about an order of magnitude) for inhalation exposure. For TCE on the other hand, PBPK modeling produced smaller increases in MRLs for inhalation exposure, but yielded much lower MRLs for oral exposure (by as much as a factor of 30).

BMD modeling

The most striking result of the analysis of the impact of BMD modeling on MRLs was the limited number of MRLs for which the data reported in the critical study was adequate to support the use of the BMD approach (2 out of 7). Moreover, for the two cases where BMD modeling could be applied, there were significant issues regarding the proper interpretation of the BMD modeling results. In both cases it was necessary to exclude the highest dose from the BMD analysis in order to obtain an adequate fit of the models to the data. In one case, only a single treated group plus control remained; it is difficult to consider the case of a single treated group plus a control to be informative regarding the dose-response below the treated dose. Another problem in the analysis of the Dawson et al. (1993) study was the fact that the data were not reported by litter, which is the most appropriate way of describing the results of a developmental study if they are to be used for dose-response analysis.

PRELIMINARY EVALUATION OF ADDITIONAL CHEMICALS

An unexpected outcome of the evaluation of the MRLs for MC and TCE was the small number of instances in which the BMD modeling approach could be applied. In order to better evaluate the generality of the potential application of both BMD and PBPK techniques for the development of MRLs, ten additional chemicals of interest to ATSDR were evaluated regarding their suitability for BMD modeling as well as regarding the availability and potential impact of pharmacokinetic models.

Results

Aldrin

No PBPK models have been published for aldrin, although a rodent model has been published for its primary metabolite, dieldrin. The most likely impact of pharmacokinetics on the oral MRLs for aldrin would be to lower the MRL due to a longer half-life for clearance in the human. The data in the critical study for the acute MRL is adequate to support BMD modeling, which could be used to estimate a NOAEL in place of the LOAEL/10. The data in the critical study for the chronic MRL are not adequate for BMD modeling because no standard deviations are reported.

Dieldrin

A preliminary pharmacokinetic model has been published for dieldrin in the rodent and the human (Lindstrom et al., 1974). The most likely impact of pharmacokinetics on the oral MRLs for dieldrin would be to lower the MRL due to a longer half-life for clearance in the human. The data in the critical study for the acute MRL is adequate to support BMD modeling, which could be used to estimate a NOAEL in place of the LOAEL/10. The data in the critical study for the intermediate MRL is not adequate for BMD modeling because no standard deviations are reported. It would take additional investigation to determine whether the data in the critical study for the chronic MRL could be used for BMD modeling, and the impact of BMD modeling is uncertain.

Inorganic Arsenic

PBPK models are available for arsenic in both rodents and humans (Buser et al., 1994, Mann et al., 1996), but it is unlikely that pharmacokinetics would have a significant impact on the MRL, which is based on human data with a very small uncertainty factor. The data in one of the the critical studies (Tseng, 1977) are adequate for BMD modeling to refine the NOAEL estimate.

Cadmium

Human PBPK models for cadmium have been published (Kjellstrom and Nordberg, 1978, 1985; Oberdoerster, 1989, 1990). However, while pharmacokinetic modeling would be useful for evaluating the consistency of various studies, it would not have a significant impact on an MRL based on a particular epidemiological study. BMD modeling might be useful to provide a more accurate estimate of the NOAELs in the epidemiological studies.

Carbon Tetrachloride

PBPK models have been developed for carbon tetrachloride in both the rodent and the human (Paustenbach et al., 1987). The impact of pharmacokinetics would probably be to increase the MRLs due to the inverse body surface area scaling of metabolism across species. The data in the critical studies for the carbon tetrachloride MRLs are inadequate for BMD modeling.

Chlordane

No PBPK models have been published for chlordane. The impact of pharmacokinetics on the MRLs for chlordane is uncertain. The data in the critical studies for the MRLs appear to be adequate for BMD modeling.

Chloroform

PBPK models have been developed for chloroform in both the rodent and the human (Corley et al., 1990, Reitz et al., 1990). The impact of pharmacokinetics on the MRLs for chloroform would, however, probably be small. It is not likely that BMD modeling of the studies underlying the MRLs for chloroform would be useful, since the only data which is adequate for BMD is on cell proliferation, which is not necessarily a good surrogate for liver toxicity.

DDT

No PBPK models have been published for DDT. The most likely impact of pharmacokinetics on the oral MRLs for DDT would be to lower the MRL due to a longer half-life for clearance in the human. The data in the critical study for the acute MRL are not adequate for BMD modeling; the data in the critical study for the intermediate MRL may be adequate for BMD modeling, but BMD analysis is unlikely to have a major impact.

Mercury

We have developed PBPK models for both methylmercury and inorganic mercury for the rodent and the human (Gearhart et al., 1995b, Andersen et al., 1995b). It is possible that the use of pharmacokinetics could improve the cross-species scaling for the mercury vapor acute inhalation MRL and the inorganic mercury MRLs. Several studies have adequate data to support BMD modeling. The use of BMD analysis to estimate NOAELs for the acute and intermediate organic mercury MRL and the acute and chronic metallic mercury MRLs would be particularly beneficial.

Tetrachloroethylene (PERC)

PBPK models have been developed for PERC in both the rodent and the human (Gearhart et al., 1993). The impact of pharmacokinetics on the MRLs for PERC would probably be small, although the oral MRL might be lower due to slower clearance in the human. The data in the critical studies for the MRLs are not adequate for BMD modeling.

Summary: Additional Chemicals

Of the 32 MRLs evaluated, only 17 are based on data that could support a BMD analysis, and for most of those the BMDL may not be very useful because of limitations in the data. PBPK models exist for 7 of the 10 chemicals evaluated. There are about 21 MRLs for which a PBPK model might be usable, but there are only a few where use of a PBPK model would be likely to effect the MRL. Of the chemicals without PBPK models, considering pharmacokinetics could lead to a lower (more conservative) MRL in many cases, particularly for oral exposure (see discussion below on general principles of cross-species extrapolation).

DISCUSSION

Utility of PBPK and BMD modeling

There are two comments which should be made regarding the use of PBPK modeling for an MRL. First, a full PBPK model may not be necessary to support a pharmacokinetic risk assessment; often only a simple compartmental pharmacokinetic description is needed; e.g., in the case of cadmium (Oberdoerster, 1989, 1990). Second, pharmacokinetics will usually not have any impact on an MRL if there is not a need to extrapolate from the critical study to obtain the MRL. Pharmacokinetics will typically only affect noncancer risk assessments which involve animal-to-human or route-to-route extrapolation. In general, then, compounds whose RfCs or MRLs are based on animal data would generally provide better candidates for the use of PBPK modeling.

An important application of PBPK modeling, which was not addressed in this study, is for route-to-route extrapolation. With a PBPK model it is possible to derive a guideline from a study performed by a different route of exposure. For example, in the case of MC, the data on the chronic oral toxicity of MC could have been used to derive a chronic inhalation MRL using the PBPK model. Similarly, the acute and intermediate inhalation toxicity data could have been used to derive acute and

intermediate oral MRLs. The recent noncancer risk assessment we performed for the EPA includes the derivation of a candidate inhalation RfC based on a dietary study in animals (Clewell et al., 1995b); this risk assessment is currently undergoing review as part of the EPA's IRIS Pilot Project.

With regard to the usefulness of BMD modeling, the greatest impact will be obtained for MRLs based on LOAELs when there is adequate dose-response data to support estimation of a NOAEL with the benchmark method, or for MRLs based on epidemiological studies for which individual exposure and response data is available.

Multiple Cross-Species Defaults

One of the general observations which grew out of this study was that there is a need for multiple defaults for cross-species extrapolation based on the nature of the chemical form producing the toxicity. Currently all chemicals are implicitly treated as if the observed toxicity is produced directly by the parent chemical itself (Andersen et al., 1995c). This implicit assumption that the parent chemical is directly toxic is true even of the new RfC dosimetry guidelines (USEPA, 1994), which differentiate respiratory effects from extra-respiratory effects and include different defaults for chemicals within these categories based on their solubility and reactivity. It should be obvious from the results presented above for MC and TCE that the correct approach for cross-species dosimetry depends on whether the toxicity is due to the parent chemical or a metabolite, and in the case of toxicity from a metabolite, whether the metabolite is reactive or stable. Moreover, the nature of the cross-species scaling for each of these possibilities is different for oral exposure than for inhalation. Therefore, a scheme for cross-species dosimetry needs to be developed which follows the approach in the RfC dosimetry guidelines, but which is expanded to include the nature of the toxic entity and the route of exposure.

To demonstrate the importance of such a scheme, the models used in this study for MC and TCE, along with a model for vinyl chloride (VC) developed for the EPA and OSHA (Clewell et al., 1995b,c), were exercised to determine general expectations for one class of chemicals, the volatile lipophilic solvents. All three of these chemicals would be considered Category 3 gases (relatively water-insoluble chemicals which achieve a steady-state during inhalation exposure) in the EPA dosimetry guidelines. Thus their animal-to-human dosimetry adjustment would be performed in exactly the same way, based on equal time-weighted average exposure concentration for inhalation

and mg/kg/day administered dose for oral exposure. The results of calculating the animal-to-human dosimetry with the PBPK models are shown in Table 2. This exercise clearly demonstrates the wide disparity between the cross-species scaling of chemical toxicity mediated by reactive metabolites as compared to stable metabolites or the parent chemical itself. The results also clearly demonstrate that different expectations are appropriate for oral and inhalation exposure. In the case of volatile, lipophilic compounds, the current standard defaults for cross-species dosimetry tend to be overly conservative for toxicity produced by reactive metabolites and insufficiently protective for toxicities produced by stable metabolites. Additional studies need to be performed with other classes of chemicals (nonvolatiles, water soluble chemicals, etc.) toward the development of a general scheme for cross-species dosimetry that considers pharmacokinetics and mode of action.

Other considerations

Although this study focused on the impact of pharmacokinetics and benchmark dose analysis on MRLs, it became apparent that improvements in the accuracy of MRLs associated with the incorporation of these techniques can be overwhelmed by the most important sources of uncertainty and non-uniformity in the process: the selection of the critical study and the choice of uncertainty factors. The study by Dawson et al., 1993, which provides the basis for the intermediate oral MRL for TCE provides an example of the first problem. The MRL based on this study is orders of magnitude lower than it would be if it were based on any other study of TCE toxicity. The decision whether this study is credible therefore dominates the MRL development process in this case.

The second problem, the impact of the choice of uncertainty factors, is illustrated by a number of the MRLs discussed above. The acute inhalation MRLs for MC and TCE are both based on subjective reporting of CNS effects by workers. In both cases only a LOAEL is reported. Yet in the case of MC an uncertainty factor of 10 is applied for use of a LOAEL, while 3 is used for the same purpose in the MRL for TCE. In those cases where BMD modeling can be applied, the NOAEL can be estimated from the dose-response of the actual toxicity data rather than applying a subjective uncertainty factor to the LOAEL. In many cases where such data exist, such as the Arito et al. 1994 study, BMD modeling has suggested that the NOAEL is much closer to the LOAEL than a factor of 10. Unfortunately, having data of the necessary quality to support BMD modeling appears to be the exception rather than the rule.

The multiplication of individual uncertainty factors, each intended to provide a margin of safety in response to a different area of concern, is a continuing problem in non-cancer risk assessment. Individual factors which might each seem reasonable in themselves, when multiplied together often produce an unreasonable total safety margin. This is of particular concern when dealing with chemicals for which there is some level of pharmacokinetic and mechanistic data. In most cases, generic defaults are used rather than tailoring the individual uncertainty factors to the specific evidence for that chemical. As a result, the impact of careful pharmacokinetic, mode-of-action, and dose-response analysis can be completely overwhelmed by an overly-conservative selection of safety factors.

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Table 1: Comparison of MRLs Using PBPK
Approach with Current MRLs

MRL	Current Approach	PBPK Approach	Ratio PBPK / Current
METHYLENE CHLORIDE			
Inhalation			
Acute	0.4 ppm	0.8-6 ppm	2 - 15
Intermediate	0.03 ppm ^a	0.2 ppm ^b	6.7
Oral			
Chronic	0.06 mg/kg/d ^a	0.13 mg/kg/d ^b	2.2
TRICHLOROETHYLENE			
Inhalation			
Acute	2 ppm	2-8 ppm	1 - 4
Intermediate	0.1 (0.04) ^c ppm ^b	0.1-0.25 ppm ^b	1 - 2.5 (2.5 - 6) ^c
Oral			
Acute	0.5 mg/kg/d ^a	0.015-0.05 mg/kg/d ^b	0.03 - 0.1
Intermediate	0.002 mg/kg/d ^a	0.0002-0.0004 mg/kg/d ^b	0.1 - 0.2

^a Uses UF=10 for animal-to-human extrapolation.

^b Uses UF=3 for animal-to-human extrapolation.

^c Corrected (using Category 3 gas calculation rather than Category 2)

Table 2: Human Equivalent Concentrations Based on Pharmacokinetic Dose Metrics for Three Volatile Chemicals¹ as Compared to EPA RfC/RfD Default Dosimetry

Inhalation Exposure

- Toxicity Due to Parent Chemical Exposure (MC, TCE, VC):
 - PBPK HEC *similar* to RfC default²
- Toxicity Due to Reactive Metabolite (MC, VC):
 - PBPK HEC 5- to 25-fold *higher* than RfC default
- Toxicity Due to Stable Metabolite (TCE):
 - PBPK HEC *similar* to 10-fold *lower* than RfC default

Oral Exposure

- Toxicity Due to Parent Chemical Exposure (MC, TCE, VC):
 - PBPK human equivalent dose 10- to 100-fold *lower* than RfD default³
- Toxicity Due to Reactive Metabolite (MC, VC):
 - PBPK human dose *similar* to RfD default
- Toxicity Due to Stable Metabolite (TCE):
 - PBPK human dose 15- to 60-fold *lower* than RfD default

¹ Based on PBPK model calculations for methylene chloride (MC), trichloroethylene (TCE), and vinyl chloride (VC).

² Default Human Equivalent Concentration (HEC) is based on equal time-weighted-average (TWA) exposure concentration.

³ Default human equivalent dose is based on equal delivered dose in mg/kg/day.

Figure Captions

- Figure 1. Fit of polynomial model to data from Arito et al., 1994: (a) Using delivered dose (mg/kg/d), (b) Using PBPK dose metric for TCE exposure (AUCB).
- Figure 2. Fit of polynomial model to data from Arito et al., 1994, omitting highest dose: (a) Using delivered dose (mg/kg/d), (b) Using PBPK dose metric for TCE exposure (AUCB).
- Figure 3. Fit of polynomial model to data from Dawson et al., 1993 for dosing during pregnancy only: (a) Using delivered dose (mg/kg/d), (b) Using PBPK dose metric for TCE exposure (AUCB), (c) Using PBPK dose metric for TCA exposure (AUC TCA).
- Figure 4. Fit of polynomial model to data from Dawson et al., 1993 for dosing before and during pregnancy: (a) Using delivered dose (mg/kg/d), (b) Using PBPK dose metric for TCE exposure (AUCB), (c) Using PBPK dose metric for TCA exposure (AUC TCA).
- Figure 5. Fit of polynomial model to data from Dawson et al., 1993 for dosing during pregnancy only, omitting high dose: (a) Using delivered dose (mg/kg/d), (b) Using PBPK dose metric for TCE exposure (AUCB), (c) Using PBPK dose metric for TCA exposure (AUC TCA).
- Figure 6. Fit of polynomial model to data from Dawson et al., 1993 for dosing before and during pregnancy, omitting high dose: (a) Using delivered dose (mg/kg/d), (b) Using PBPK dose metric for TCE exposure (AUCB), (c) Using PBPK dose metric for TCA exposure (AUC TCA).

Figure 1(a)

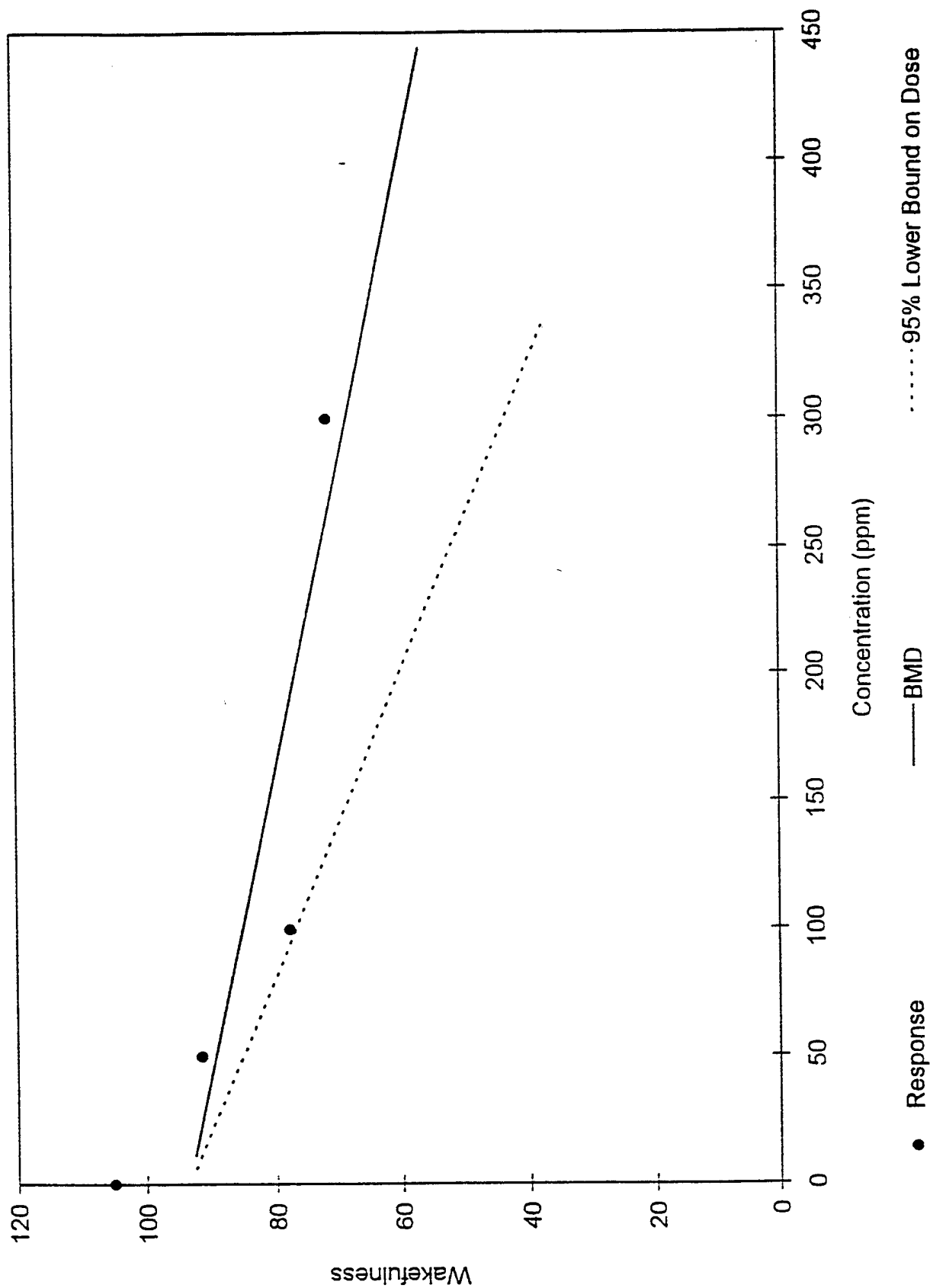


Figure 1(b)

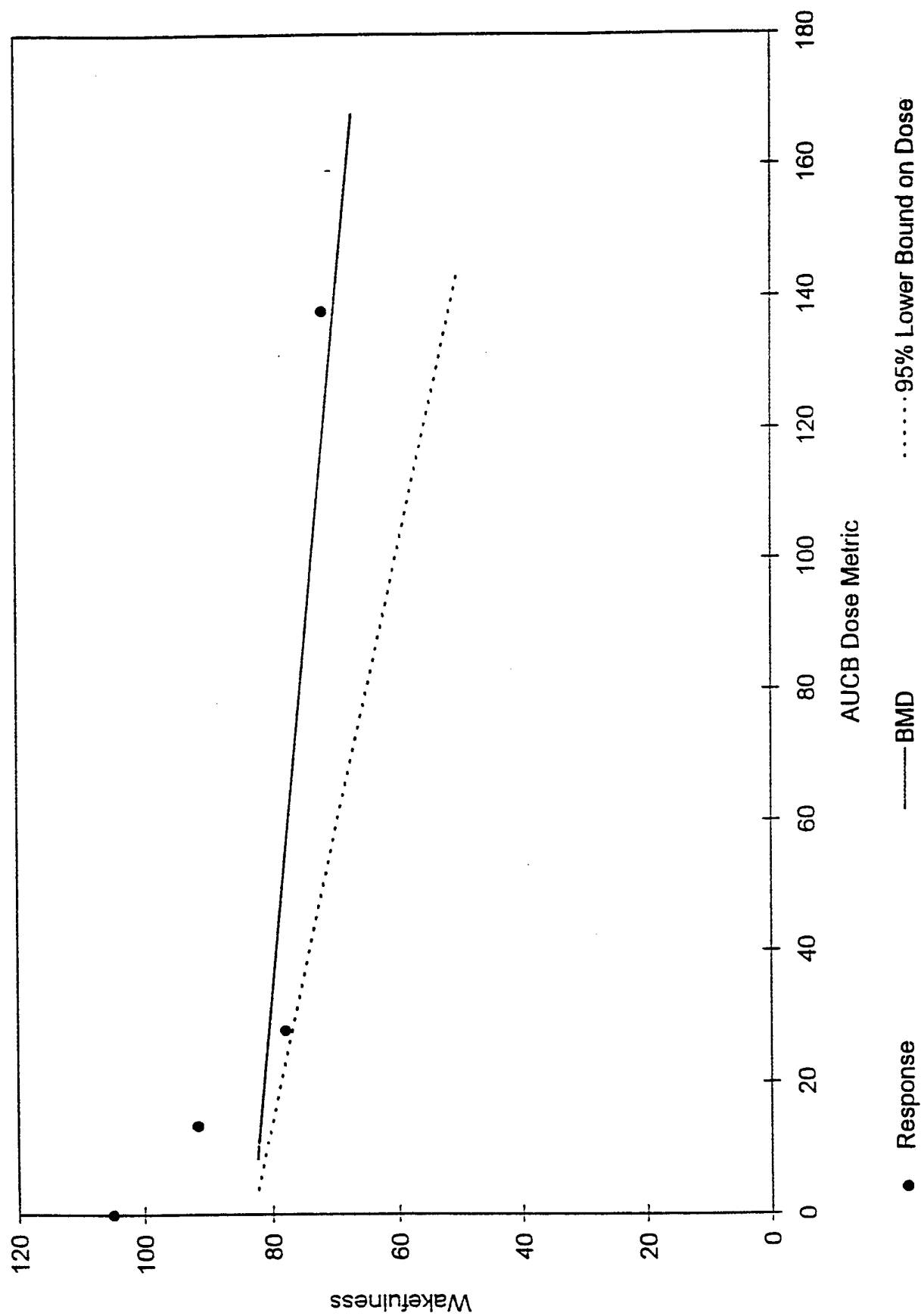


Figure 2(a)

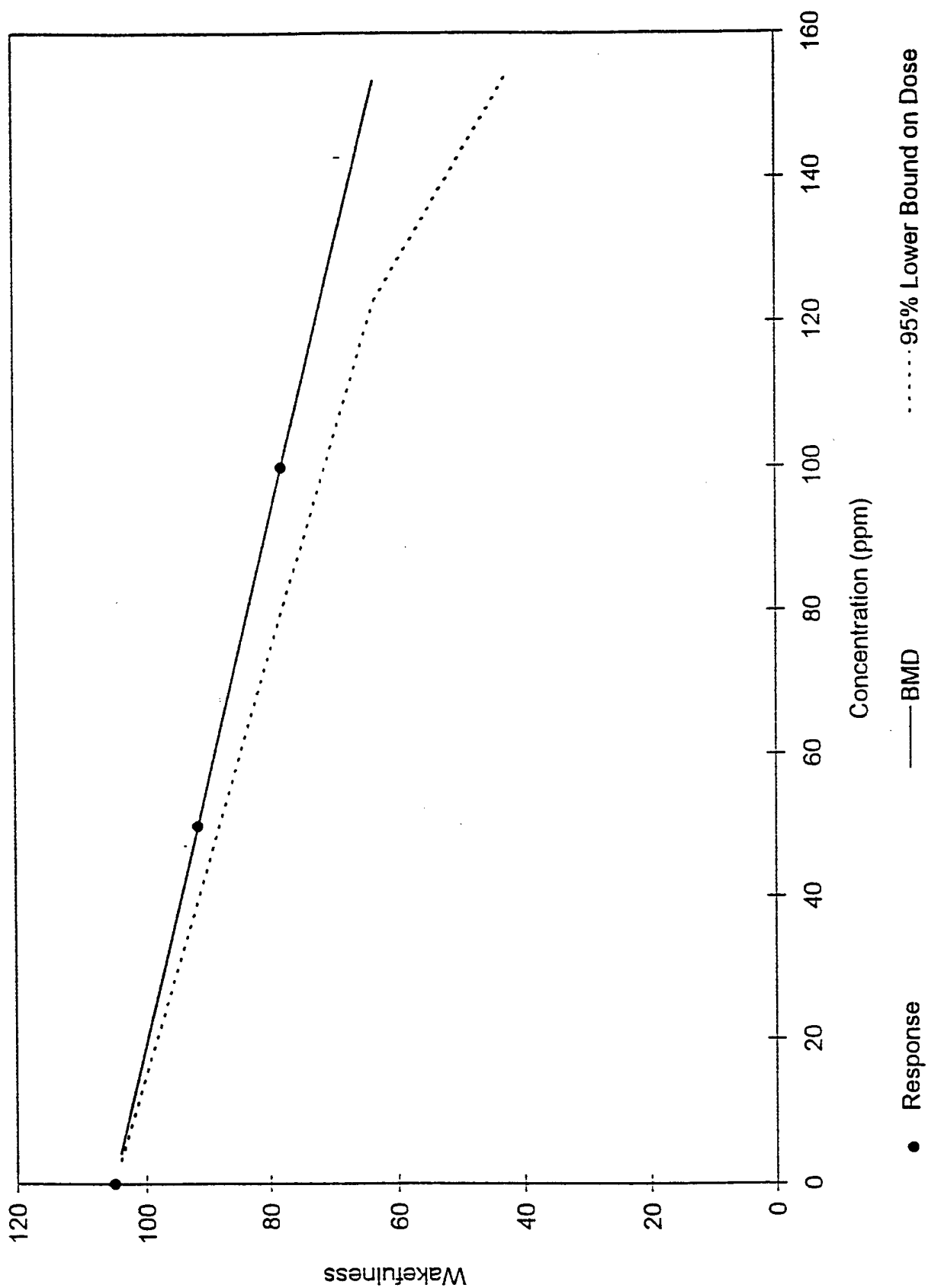


Figure 2(b)

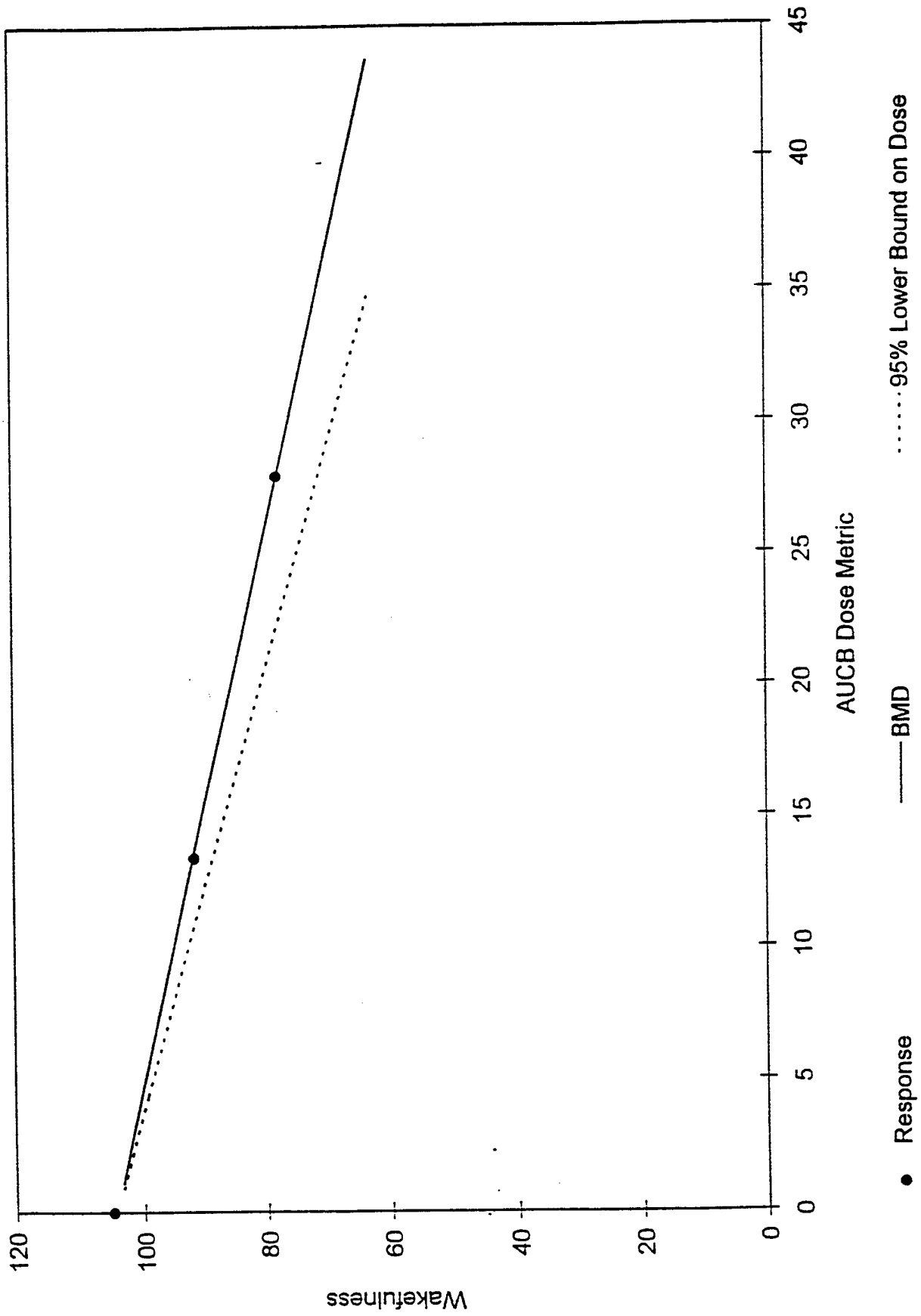


Figure 3(a)

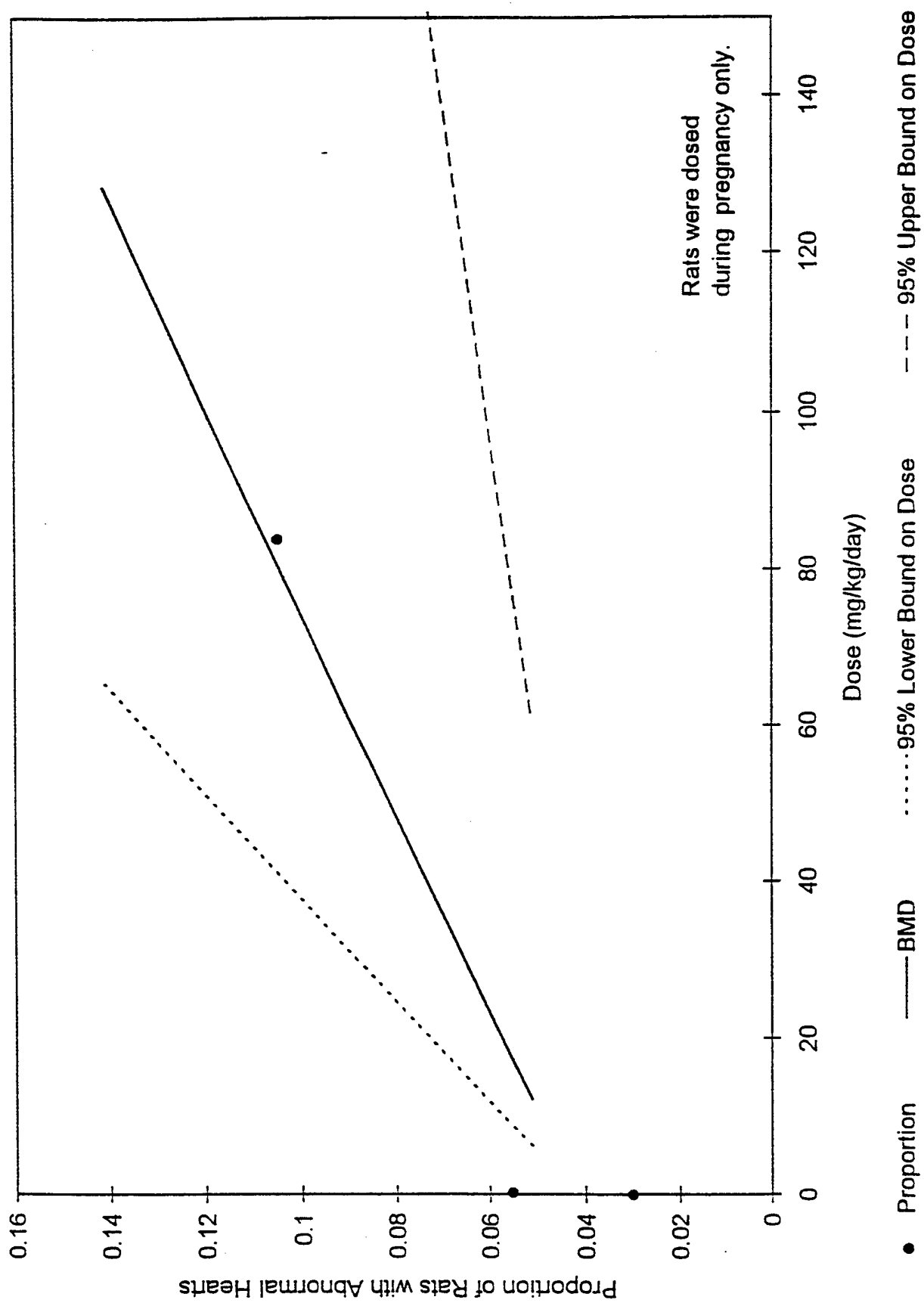


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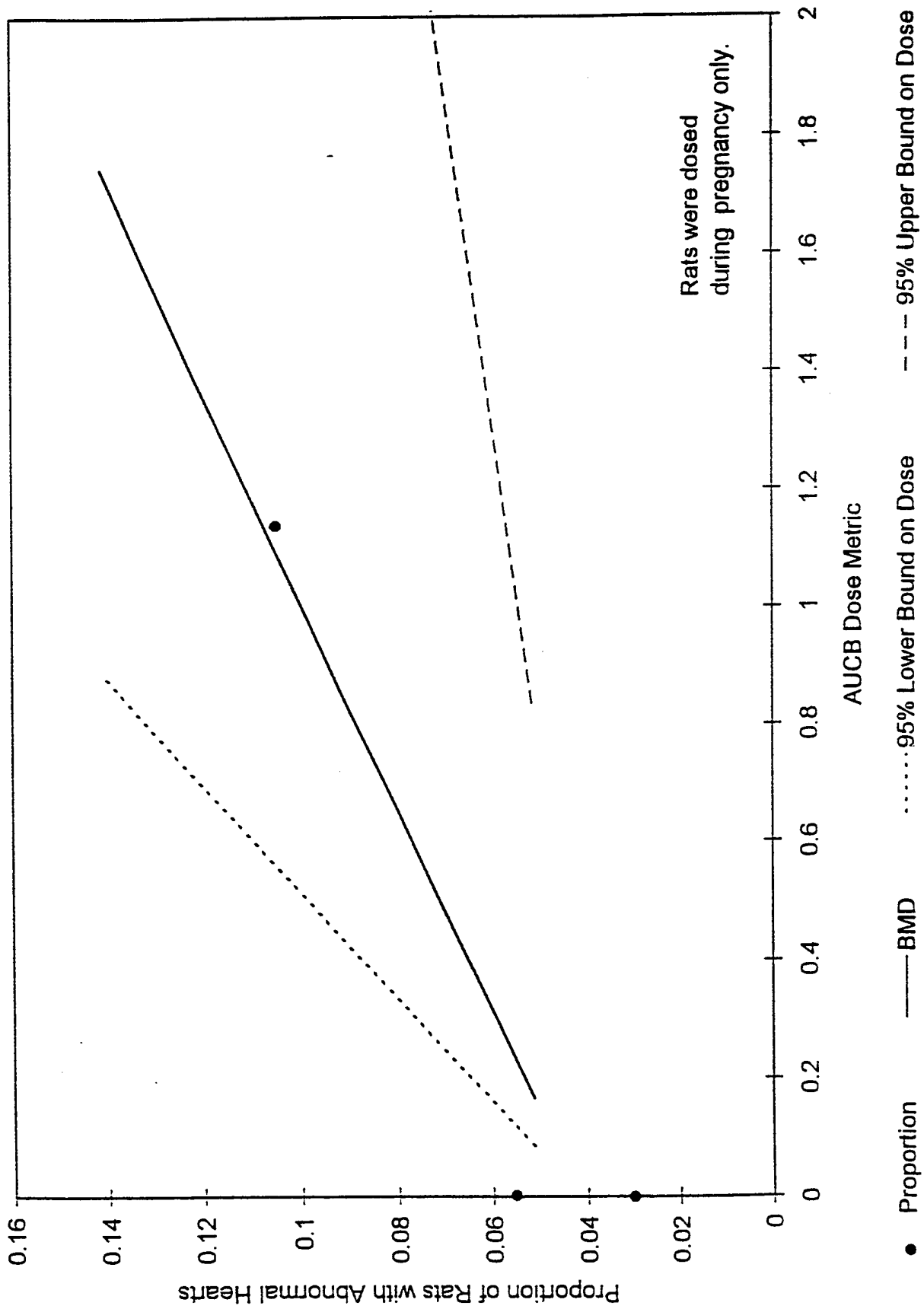


Figure 3(c)

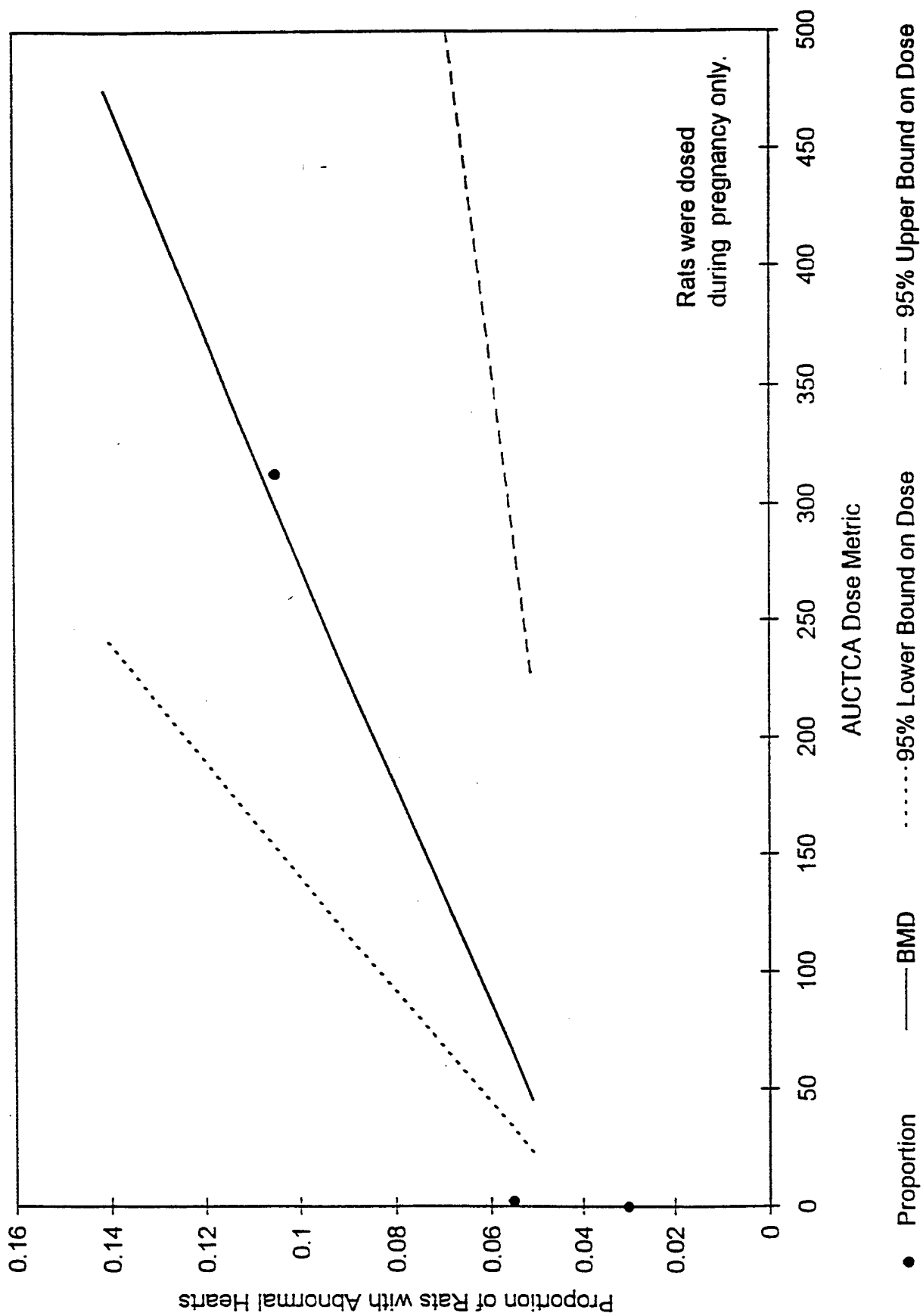


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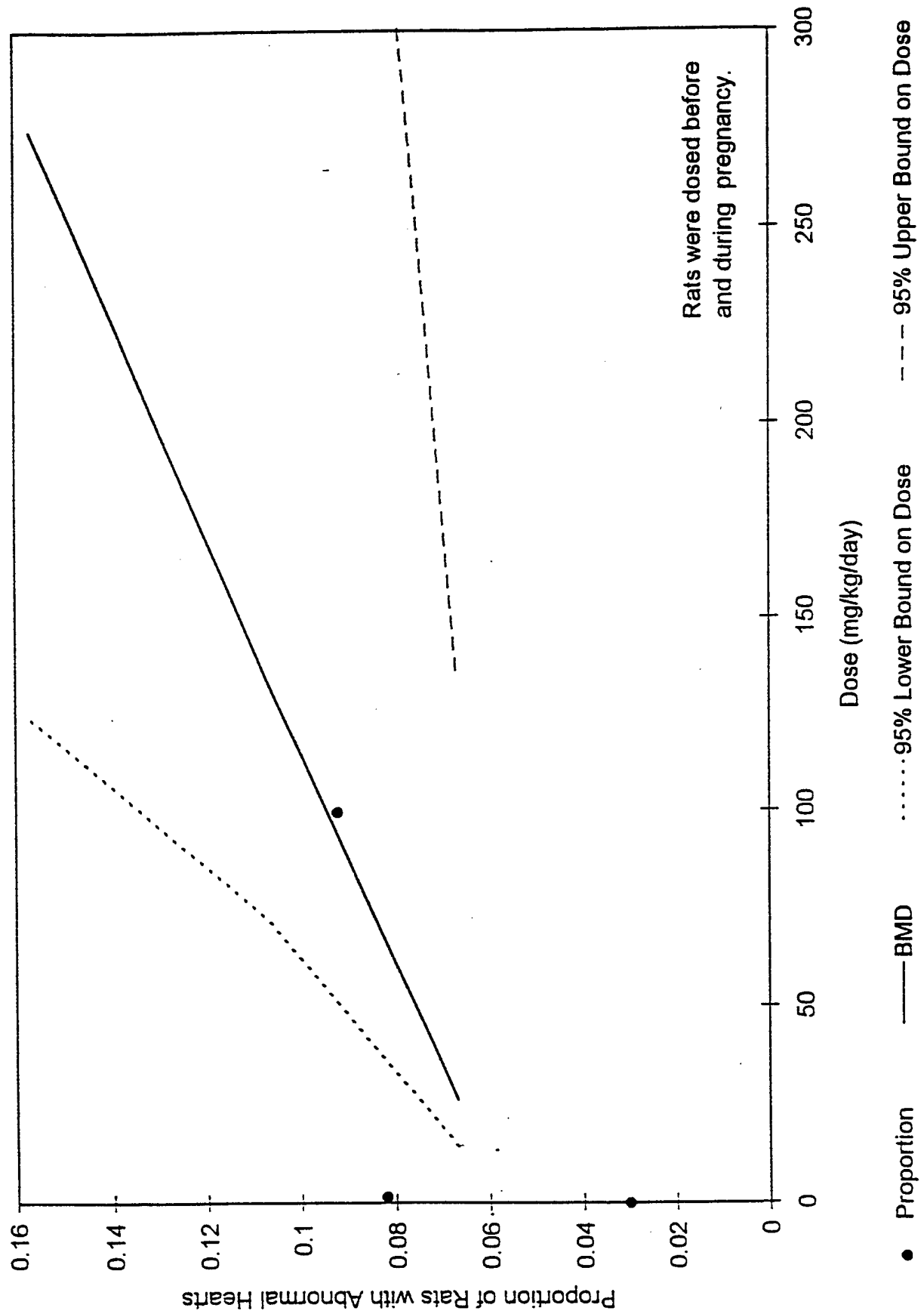


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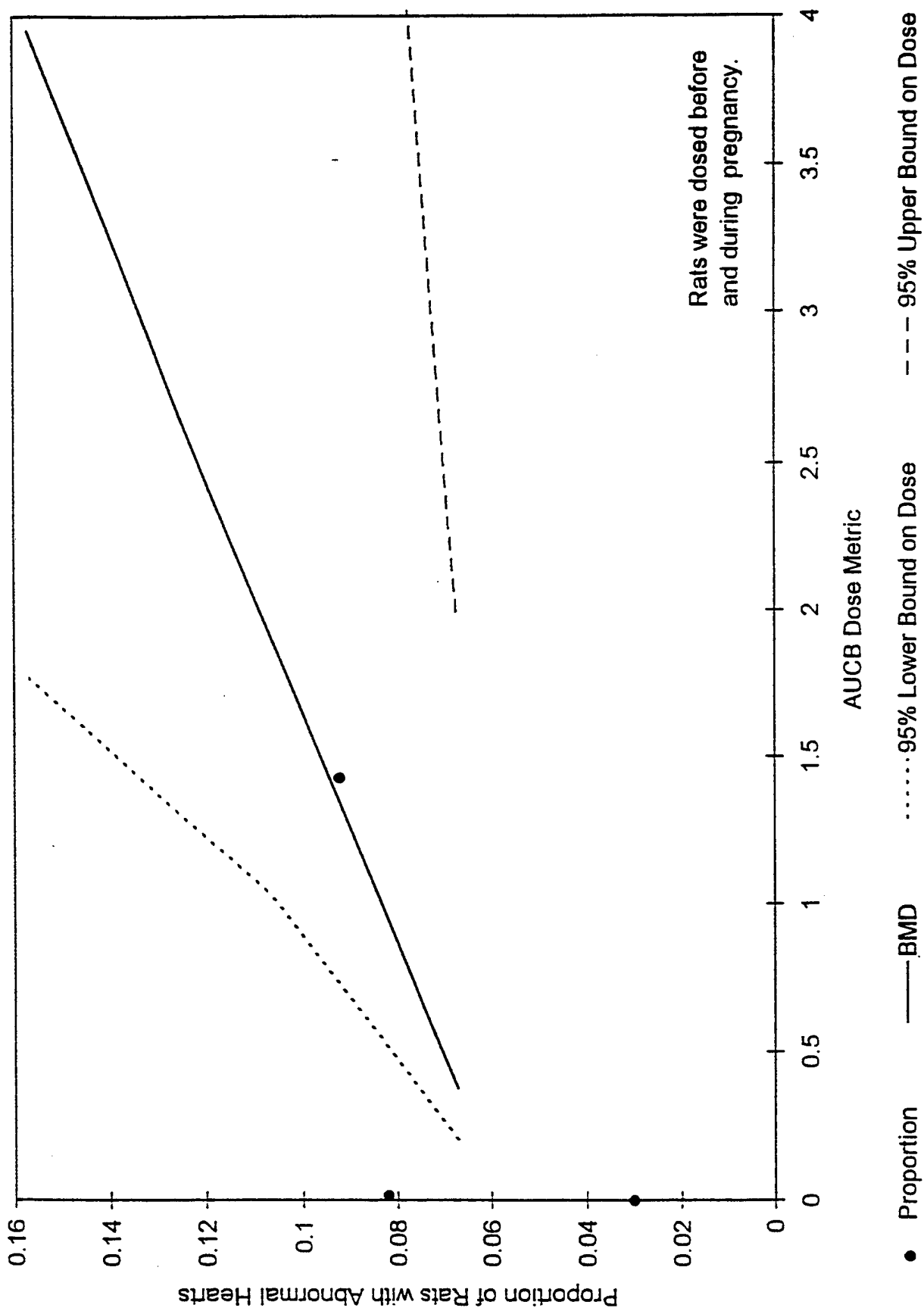


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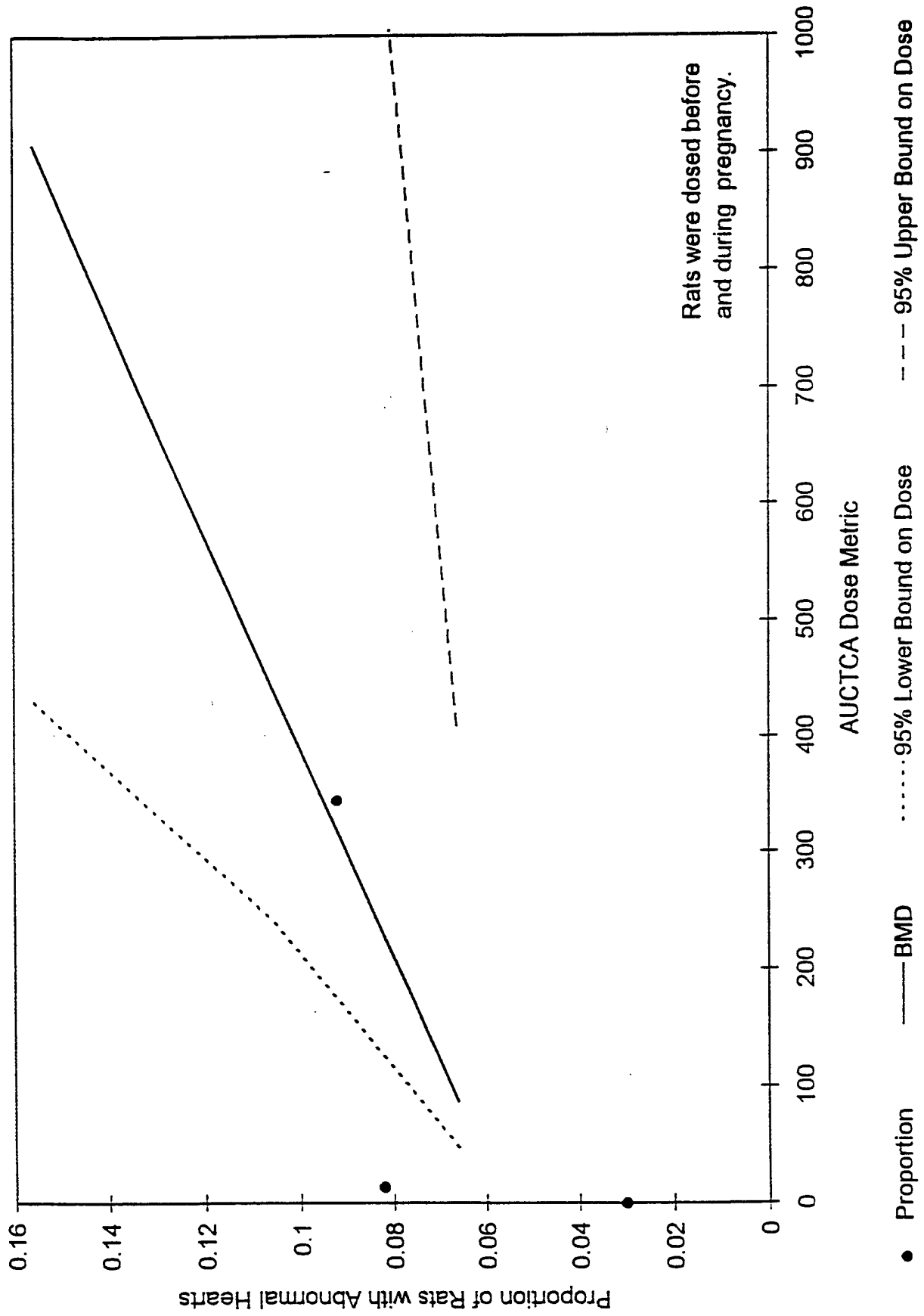


Figure 5(a)

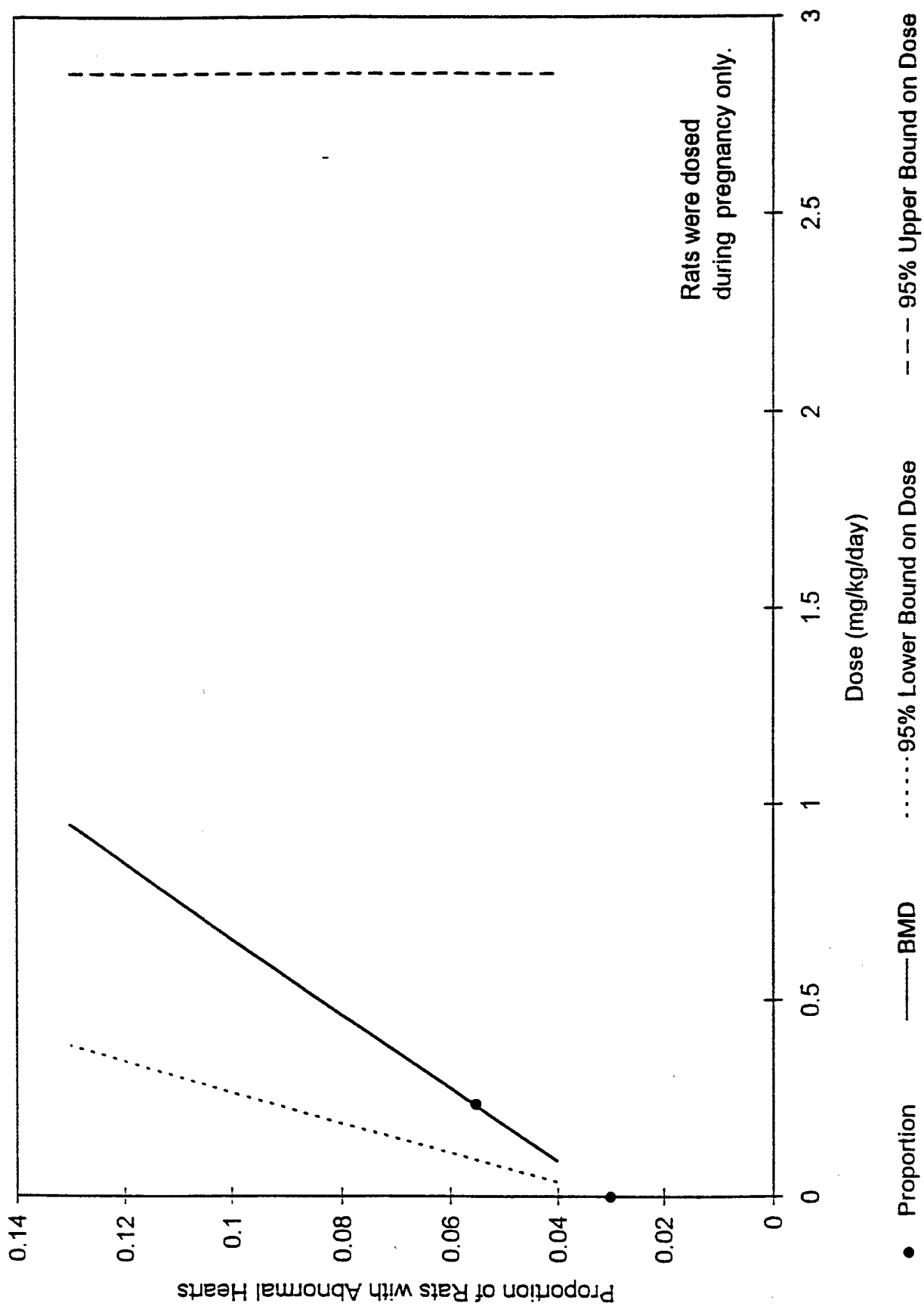


Figure 5(b)

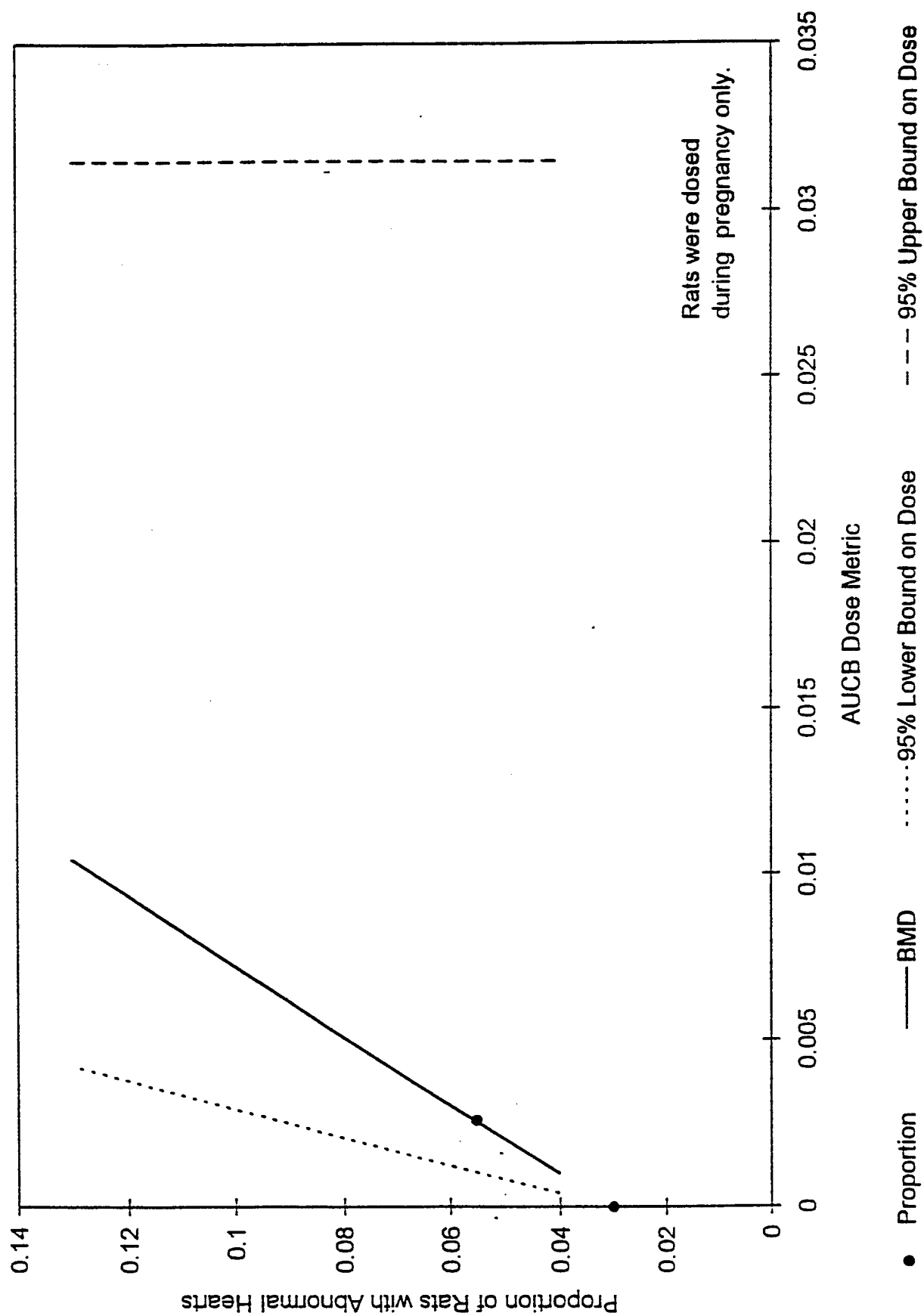


Figure 5(c)

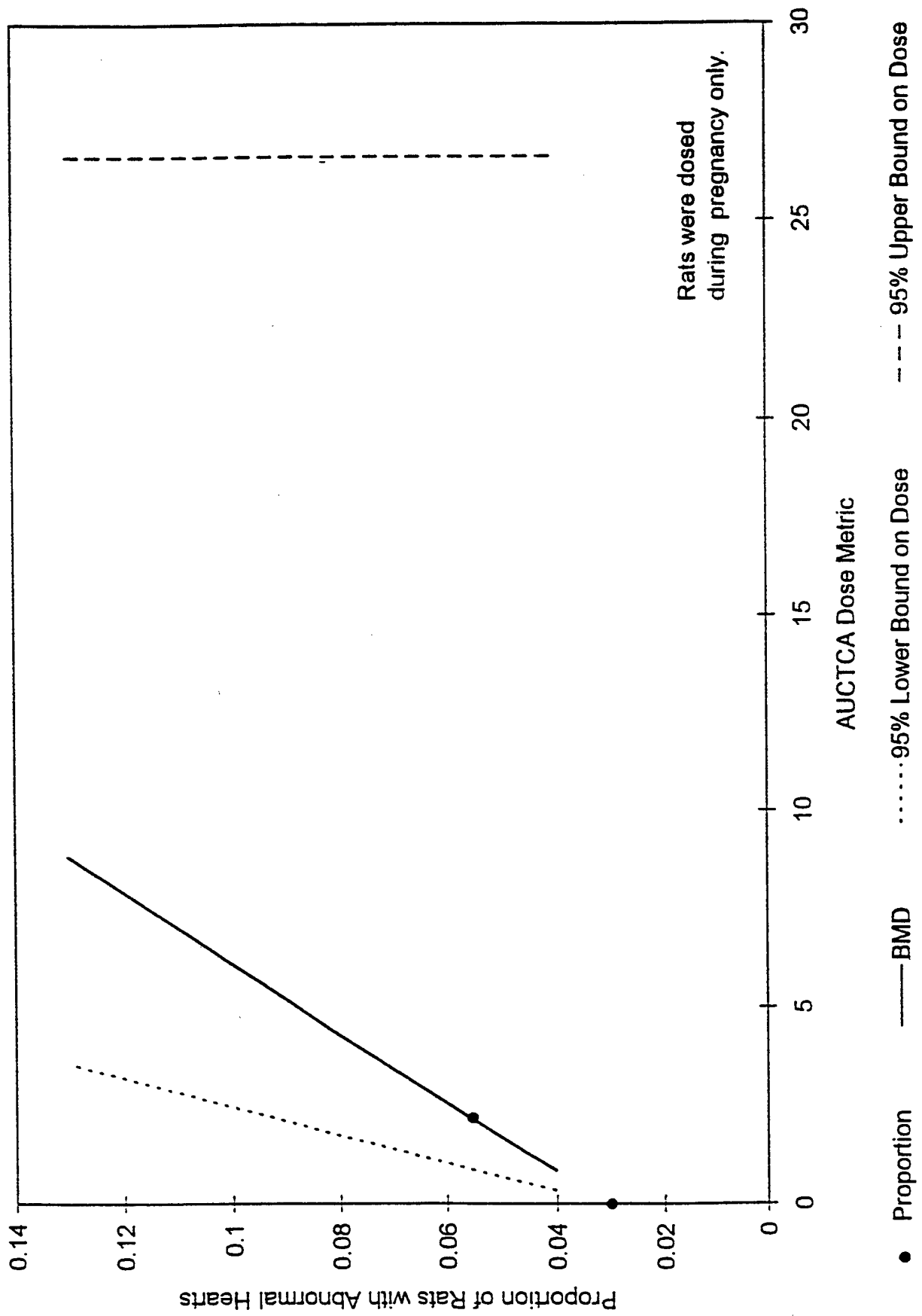


Figure 6(a)

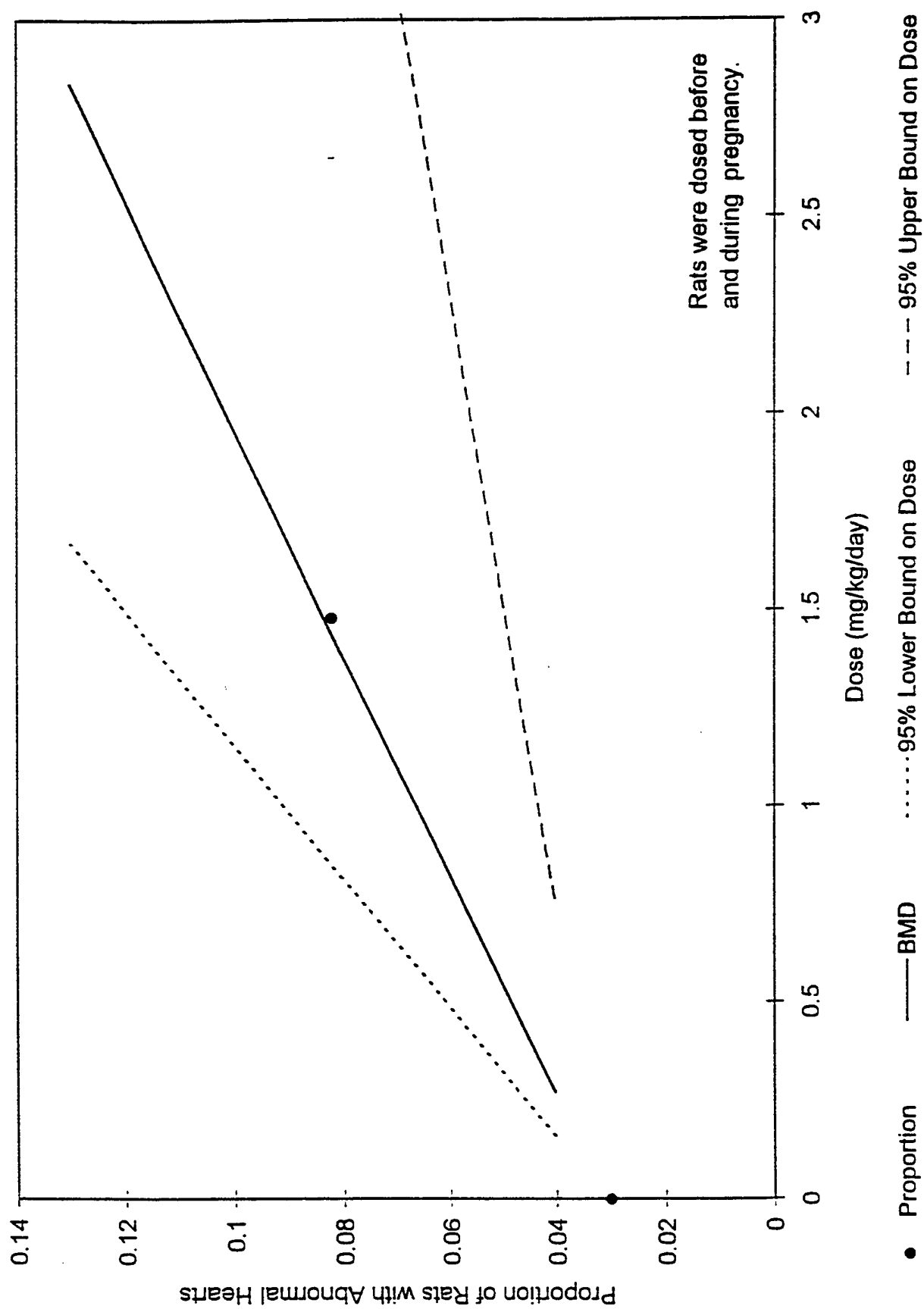


Figure 6(b)

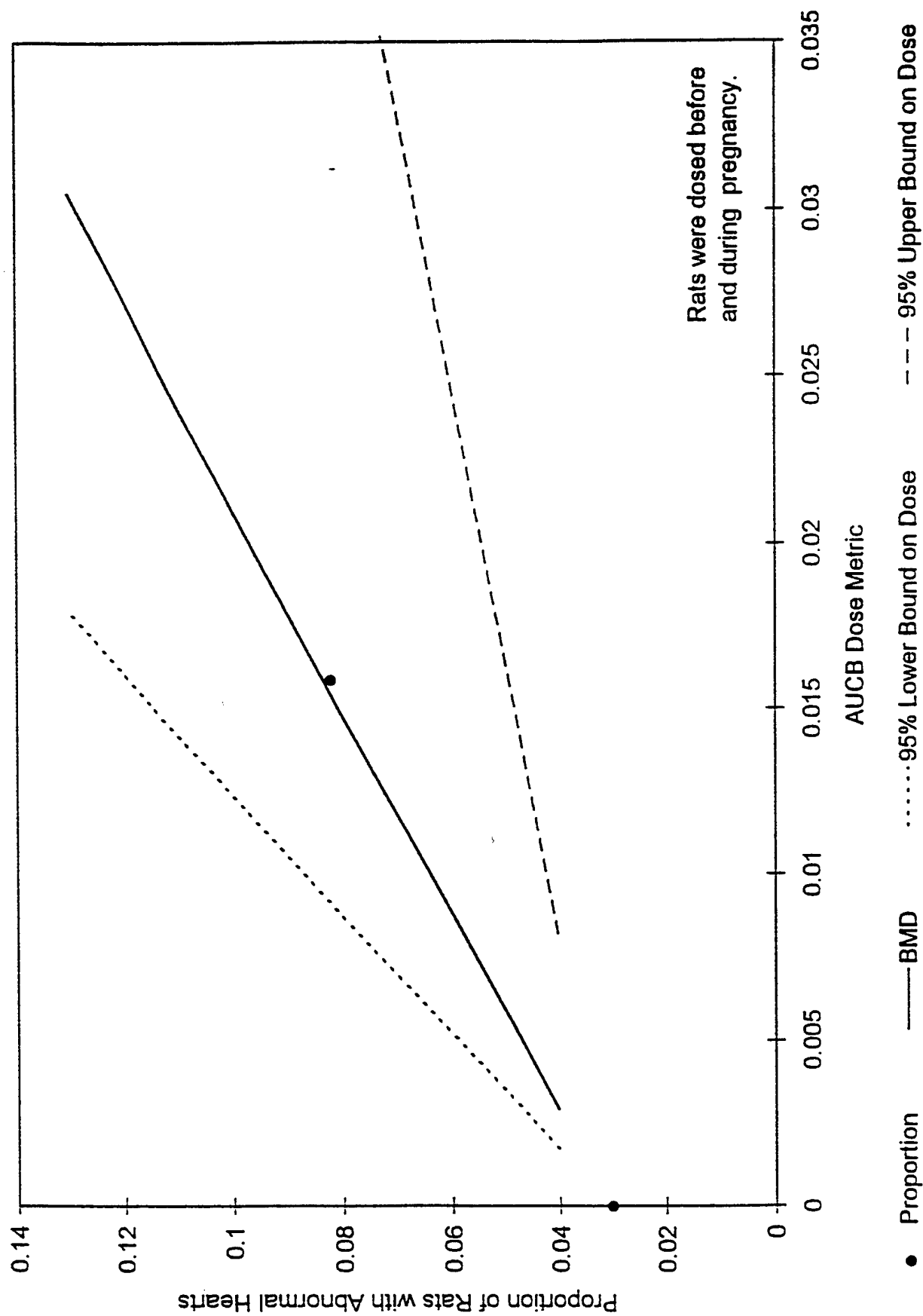
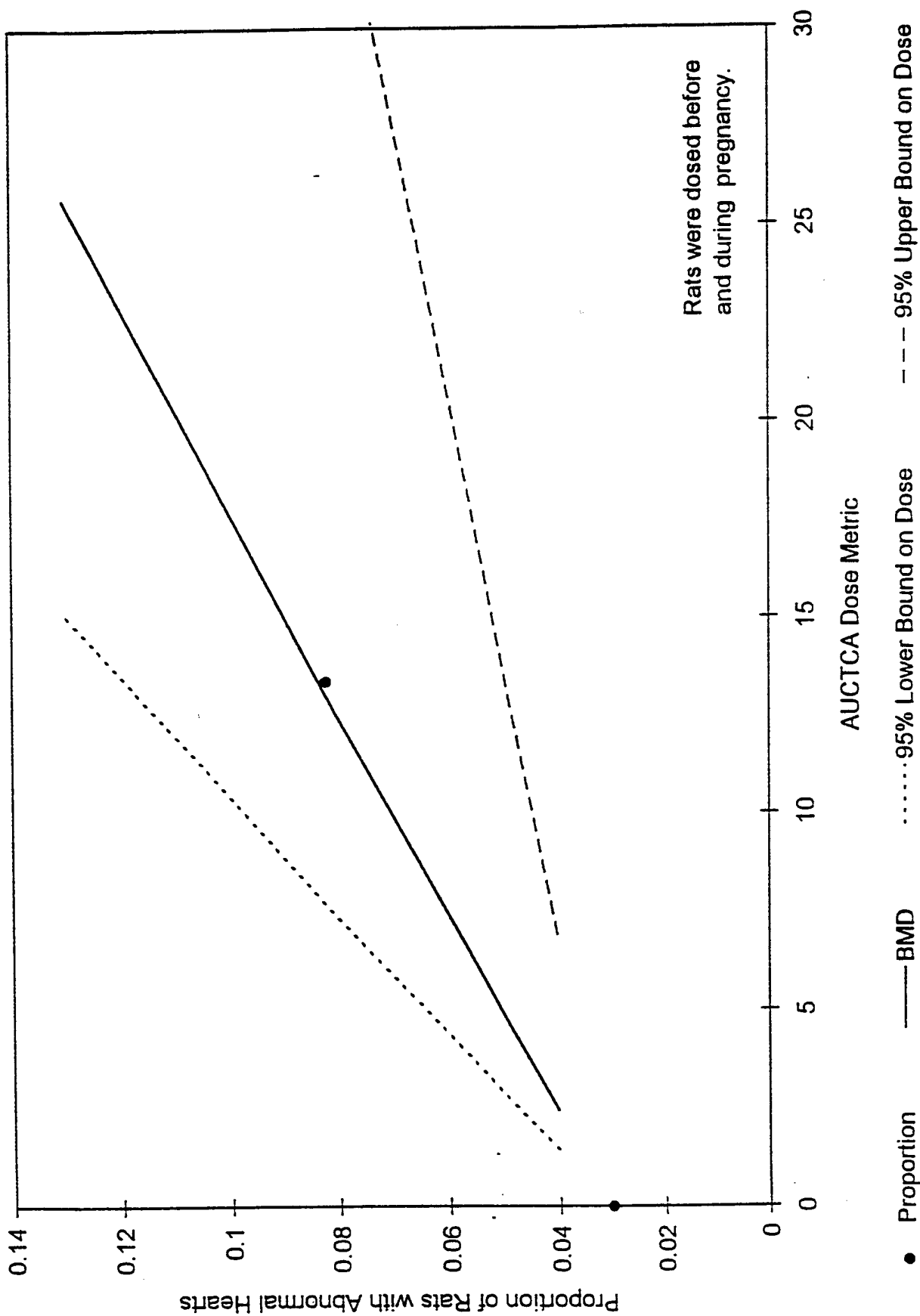


Figure 6(c)



**ABSTRACT OF PRESENTATION:
BROMODICHLOROMETHANE: CANCER
RISKS, MECHANISMS, AND MODELING
APPROACHES**

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Bromodichloromethane (BDCM), a by-product of water chlorination, is generally second only to chloroform (CHCl_3) in prevalence among disinfection by-products in finished drinking water. In chronic studies, BDCM appears to be a more potent carcinogen in rodents than CHCl_3 , producing tumors at lower daily doses in the liver, kidney, and large intestine. Carcinogenic responses induced by BDCM are likely dependent on the amount of compound reaching target tissues and the rates of metabolism to reactive intermediates. Research has been undertaken to examine the pharmacokinetics and biological activities of BDCM in rats with the goal of developing a biologically based dose response model for BDCM carcinogenesis. Comparisons to CHCl_3 have been incorporated into experiments when possible. A physiologically based pharmacokinetic (PBPK) model has been developed for BDCM in male F-344 rats using circulating bromide levels after constant concentration inhalation exposures to determine metabolic rate constants. Parameterization of the oral uptake of BDCM was accomplished by fitting blood and exhaled breath chamber BDCM concentration-time profiles using gut sub-compartment absorption constants, bioavailability terms and emptying times. Model simulations were in good agreement with measured target tissue BDCM levels following oral BDCM administration. The metabolic parameters and partition coefficients derived for the model as well as various model simulations indicate that BDCM is absorbed into tissues and metabolized more rapidly than CHCl_3 and that metabolism other than oxidation may

be important for BDCM. Two saturable pathways, one high affinity and one low affinity, have been included in the PBPK model description of BDCM metabolism. The cytochrome P450 isozymes, CYP2E1 and CYP2B, are involved in the metabolic activation of BDCM, which is required for hepatotoxicity. Two potential carcinogenic modes of action have been examined for BDCM: cytotoxicity and genotoxicity. The cytotoxicity of THMs has been attributed to macromolecular binding, and *in vitro* microsomal protein and lipid binding by metabolites of BDCM was found to exceed that of CHCl_3 -derived intermediates. More rapid generation of toxic intermediates from BDCM, as predicted by the PBPK model and the *in vitro* assays, may explain the greater acute *in vivo* renal cytotoxicity and more persistent hepatotoxicity of BDCM compared to CHCl_3 that has been observed in rats. To better characterize BDCM genotoxicity, we employed a strain of *S. typhimurium* TA1535 transfected with glutathione S-transferase (GST) (Thier *et al.*, 1993, *Proc. Natl. Acad. Sci.*, 90: 8576) to demonstrate that BDCM can be activated to a mutagenic intermediate via glutathione (GSH) conjugation, while CHCl_3 was found to be inactive in this assay system over a similar concentration range. These results demonstrate that GSH-mediated activation of THMs is possible and may explain the greater genotoxicity of brominated THMs compared to CHCl_3 . Moreover, the theta-class GST which catalyzes this reaction is present in humans and is expressed polymorphically. Significant *in vivo* covalent binding of BDCM to DNA was observed in all cancer target tissues 6 hr after oral dosing of rats, but was especially high in the liver and intestinal tract. Our results in conjunction with data from other studies demonstrate that chloroform is not the most potent THM in drinking water. BDCM exhibits greater carcinogenicity, metabolic rates, macromolecular binding, and both cytotoxicity and genotoxicity. These findings further indicate that the different

THMs do not act to produce cancer via identical mechanisms, and a BBDR model for BDCM carcinogenesis should include both cytotoxic and genotoxic modes of action.

(This abstract does not necessarily reflect EPA policy.)

PHYSIOLOGICAL "CONSTANTS" FOR PBPK MODELS FOR PREGNANCY

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ABSTRACT

Physiologically-based pharmacokinetic (PBPK) models for pregnancy are inherently more complex than conventional PBPK models due to the growth of the maternal and embryo/fetal tissues. Physiological parameters such as compartmental volumes or flow rates are relatively constant at any particular time during gestation when an acute experiment might be conducted, but vary greatly throughout the course of gestation (e.g., contrast relative fetal weight during the first month of gestation with the ninth month). Maternal physiological parameters change during gestation, depending upon the particular system; e.g., cardiac output increases by ~50% during human gestation; plasma protein concentration decreases during pregnancy; overall metabolism remains fairly constant. Maternal compartmental volumes may change by 10-30%; embryo/fetal volume increases over a billion fold from conception to birth. Data describing these physiological changes in the human are available from the literature. Human embryo/fetal growth can be well described using the Gompertz equation.

By contrast, very little of these same types of data are available for the laboratory animal. In the rodent there is a dearth of information during organogenesis as to embryo weights, and even less organ or tissue weight or volume data during embryonic or fetal periods. Allometric modeling offers a reasonable choice to extrapolate (approximately) from humans to animals; validation, however, is confined to comparisons with limited data during the late embryonic and fetal periods of development (> gestation day 11 in the rat and mouse). Embryonic weight measurements are limited by the small size of the embryo and the current state of technology.

However, the application of the Laser Scanning Confocal Microscope (LSCM) to optically section intact embryos offers the capability of precise structural measurements and computer-generated three-dimensional reconstruction of early embryos.

Application of these PBPK models of pregnancy in laboratory animal models at teratogenically sensitive periods of development provides exposure values at specific target tissues. These exposures provide fundamentally important data to help design and interpret molecular probe investigations into mechanisms of teratogenesis.

INTRODUCTION

Pharmacokinetic modeling attempts to mathematically represent the absorption, distribution, metabolism, and elimination of a xenobiotic in an intact animal. These models are based on the assumption that most transport functions obey first order kinetics and that distribution can be represented by a few fluid compartments representing all of the fluids, organs, and tissues of the animal system. For data that are restricted to blood and elimination values from a single animal, these simple models describe the data fairly well. Extrapolation or comparison between species is difficult, however, since these simple models may not be applicable to multiple species (e.g., a one-compartment model may apply for one species and a two-compartment model for another; or an additional metabolite may need to be considered in one of the species). A small alteration in the model may make a realistic comparison between species impossible.

Physiologically-based pharmacokinetic models (PBPK) are designed to describe an animal system more realistically by including specific volumes for the individual fluid, tissue, and organ masses as well as actual flow rates for transport within the body (Bischoff et al., 1971). Physiological parameters, such as volumes and flow rates, can be described for each species and usually are considered constant within a species, independent of xenobiotic exposure. The variables of the PBPK model, specific for each xenobiotic, include the partition coefficients into the various organs/tissues and the method for transport into and out of the animal (i.e., absorption and elimination, including metabolism). The partition coefficients are usually estimated from *in vitro* experiments, leaving only the absorption and elimination

parameters to be manipulated when fitting the *in vivo* experimental data using the model. Since the anatomy and physiology are similar among the various laboratory animals and humans (i.e., each animal has a heart, liver, kidney, etc.), once the volumes and flows are specified for each animal model, extrapolation between species for a xenobiotic involves only the input and output parameters. Alterations in the xenobiotic metabolism, qualitative or quantitative, are often the major difference between species and can be readily accounted for in PBPK models. For risk assessment deliberations, the restrictions and limits imposed by the model for extrapolation between species greatly simplify the process and arguments.

PBPK MODEL OF PREGNANCY

Pregnancy introduces growth or change into the normally static PBPK model. During human pregnancy a weight gain of 4-14 Kg (10 to 30 pounds) is normal (Figure 1). For example, if a 50 Kg woman increased to 62 Kg during her pregnancy, this would constitute an ~25% increase in weight. This would be the result of an ~32% increase in body water, an ~20% increase in body fat, an ~50% increase in plasma volume, and an ~36% increase in extracellular volume (Mattison et al., 1991). The effects on xenobiotic distribution by dilution alone would result in very different tissue dosimetry and plasma concentration values; simple xenobiotic clearance would also be affected.

But why is knowing the exact volume at a given time during gestation important? It's required by the mathematics! Pharmacokinetic analyses are based on differential equations which describe the change in mass (e.g., amount) of a xenobiotic (x) in an organ/tissue with a

change in time (t); i.e., dx/dt . However, blood or plasma data are always, by necessity, expressed in concentration units; i.e., amount/volume. To convert concentration to mass, a volume term is necessary: Amount = [Amt/Vol][Vol] = [Conc][Vol]. This same volume consideration must be applied to all organs and tissues in the PBPK models. In its simplest form, the differential equation for the uptake of a xenobiotic by any organ or tissue in a general PBPK model is given by

$$dx_i/dt = Q_i * D_i * (C_p - C_i/R_i) \quad (\text{Eq 1})$$

where Q_i is the flow rate, D_i is a factor for diffusion limited transport, and R_i is the binding coefficient to the i^{th} tissue, and $(C_p - C_i/R_i)$ is the difference in xenobiotic concentration between plasma and tissue (C_p is the concentration in the plasma and C_i is the concentration in the i^{th} tissue). However, in the PBPK model for pregnancy, the general format is expanded to compensate for the maternal/fetal growth that occurs:

$$d\{f_{vi}(t) * C_i\}/dt = f_{Qi}(t) * D_i * (C_p - C_i/R_i) \quad (\text{Eq 2})$$

where $f_{vi}(t)$ is the function that represents the change in volume of the i^{th} organ/tissue during gestation, and $f_{Qi}(t)$ is the function for the change in flow rate. These growth functions have been incorporated into a PBPK model for human pregnancy which can accommodate two chemical entities in 27 maternal fluids/organs/tissues and 16 embryo/fetal compartments (Luecke et al., 1994).

There are also other changes that occur during pregnancy that affect the fate of a xenobiotic in the maternal system. Cardiac output, pulmonary function, and renal blood flow all increase during pregnancy; while intestinal motility and plasma proteins decrease during

gestation (Mattison et al., 1991). All of these maternal changes affect the exposure profile of the xenobiotic to the embryo/fetus.

ASSESSING "CONSTANTS" FOR HUMAN MODELS

The maternal changes that occur during human or animal pregnancy are small when compared to the growth and changes that occur during embryonic and fetal development. From the fertilized egg to birth, gestation involves over a billion fold increase in volume for the embryo/fetus. Figure 2 illustrates this growth in the human embryo and fetus (adopted from Wosilait et al., 1992; Jackson, 1909; Hertig et al., 1956; Jirasek et al., 1966; Potter & Craig, 1975). These data represent more than 4400 specimens from four different investigations; the s-shaped curve was generated utilizing a Gompertz equation of the form:

$$W_F(t) = W_0 \exp\{(A_0 / \alpha)(1 - \exp[-\alpha(t - t_0)])\} \quad (\text{Eq 3})$$

where $W_F(t)$ is the weight of the embryo/fetus at any time t post-conception, W_0 is the mass at time t_0 , A_0 is the relative growth rate at time t_0 , α is the exponential rate of decrease in the growth rate, and t_0 is the time at which growth is initiated. Once t_0 is given or assumed, W_0 , A_0 , and α may be determined through regression analysis. While W_0 and A_0 depend upon the choice of t_0 , α is independent of t_0 . Thus α alone may be used to control the shape of the growth curve. This feature is especially useful when investigating xenobiotics that result in growth retardation as a part of a malformation scenario.

Embryonic and fetal liver growth in humans is illustrated in Figure 3 (from Luecke et al., 1995; Jackson, 1909; Potter & Craig, 1975; Shepard et al., 1988). Both linear and logarithmic

plots are included to show the goodness of fit of the regression line throughout the data set. The fitted curve is generated from the allometric equation of the form:

$$W_i = a_i W_F^{b_i} \quad (\text{Eq 4})$$

where W_i is the weight of the i^{th} organ, W_F is the weight of the embryo/fetus, and a_i and b_i are parameters determined empirically by regression for the i^{th} organ (Luecke et al., 1995).

Figure 4 illustrates human embryonic and fetal bone growth (from Luecke et al., 1995; Trotter & Peterson, 1968). The calculated curve is also obtained by the allometric equation. There are no low embryonic weight values as bone forms later in development. On the other hand, the Gompertz equation was utilized to generate the curves in Figure 5 for the development of the human embryonic/fetal femur, tibia, humerus, fibula, ulna, and radius bones. The good fit of the Gompertz equation to the embryonic total weight data over that very wide range (Figure 2) indicates that reasonable extrapolation is feasible for the bone data. The data that were fit came from tabled values of ultrasound data from Mahony (1994).

Even though the human data are extensive, there are some limitations to their exactness since they are derived mainly from aborted embryos and fetuses. The control on the timing of the data collection is thus limited and contributes to the sparseness of the data in the middle of gestation when the slope of the curve is the steepest (Figure 2). There is a lack of accuracy regarding the exact time of conception which also contributes to the error on the x-axis. The long gestation period in humans and relatively slow growth and development also contributes to the lack of an assignment of a more exact developmental time for the specimens.

ASSESSING "CONSTANTS" FOR ANIMAL MODELS

Compared to humans, the laboratory rodent offers an interesting contrast in development. The mouse has a gestational time of about 18 days and the rat 21 days. The timing of conception can be determined within a few hours, and since gestation is short, developmental landmarks are more discrete in time. Despite these advantages, however, there are still more human embryo/fetal growth data than there are laboratory animal data. One reason for this may be just heightened interest in human development over animals; another might be the extremely small size of the rodent during organogenesis which makes identifying and weighing individual organs extremely difficult if not impossible. Weighing is the key, as generally whole embryo weights are only available above gestation day (GD) 9 (Figure 6; Rugh, 1968). This is not meant to imply that knowledge of embryo/fetal morphology in rodents is lacking, for it is not. Many illustrated text books are available on both the mouse (Rugh, 1968; Kaufman, 1992) and the rat (Hebel & Stromberg, 1976) which contain detailed structural identifications from serially sectioned embryos and fetuses.

Current computer technology provides a new approach to embryonic volume calculations. Serial sections of embryos or fetuses may now be electronically imaged, and the digital data reconstructed into a 3-dimensional format (Figure 7). Organs identified using these electronic sections can be contoured (i.e., outlined) and further manipulated (i.e., isolated and reconstructed) to yield volume measurements (Figure 8). At very early embryonic ages (< GD 9) a laser scanning confocal microscope can be utilized to optically scan intact embryos to yield similar serial section data. We are in the process of collecting and imaging both CD rat and CD-

1 mouse embryos and fetuses from ~ GD 7 through term. Taken together, these data should provide an accurate assessment of organ/tissue volumes for use in PBPK models throughout all of rodent pregnancy.

WHY ARE SUCH DETAILED VOLUME MEASUREMENTS NEEDED IN RODENT EMBRYOS?

There are no known mechanisms of teratogenesis. We do not know how any xenobiotic produces a malformation in a developing embryo. We know very little about the molecular interactions at the cellular level between a xenobiotic and any organ system. However, we do know quite a bit about gestational timing, and we have dose-response data for specific teratogens. PBPK models with finely tuned organ development data can provide specific exposure profiles to help guide the molecular biologist to design and test specific probes. The organ/tissue volume data for all times during development are essential to our PBPK model in order to provide estimates of the xenobiotic mass measurements at the time of developmental sensitivity. Wilson (1973) amply illustrated the developmental sensitivity of various organ/tissue systems by showing that different organs develop at different gestational times and that the percent of the embryos/fetuses affected by a given xenobiotic changes with gestational time.

In applying pharmacokinetic principles to this sensitivity, a "critical window" concept was presented by White et al. (1980). Consider a very simple two compartment model as illustrated in Figure 9 where 'B' represents the mass of the chemical/teratogen in the maternal

system, 'T' the teratogen mass in the embryo/fetal target tissue, and 'E' the elimination of the chemical from the maternal system following a single dose of a xenobiotic. If we assume that there is some "critical amount" of chemical necessary for a malformation to occur (illustrated by the dashed line), then the shaded area represents the target tissue exposure above that critical amount; i.e., a minimal teratogenic exposure level. If we further assume that the target tissue only needs to be exposed for a specific time or duration as represented by the width of the shaded area, then Figure 10 illustrates this "critical duration" above a critical exposure amount. This logic applies to either a peak amount or area-under-the-curve (AUC) type of critical exposure. Figure 11 adds a third component of developmental sensitivity to the exposure curves indicating that peak malformations will be observed if target tissue exposure (amount and duration) occurs at any time during GD 9. For a xenobiotic to produce a teratogenic effect, all three critical criteria must be met: critical amount, critical duration, and critical gestation time. Figure 12 is included to demonstrate that if the chemical was administered just $\frac{1}{2}$ day earlier, then the critical duration of exposure (either peak or AUC) would occur outside the developmentally sensitive period, and no malformations would be observed; i.e., one of the three critical criteria was not met, and therefore malformations would not be produced.

THEORETICAL MODEL FOR LIMB DEVELOPMENT

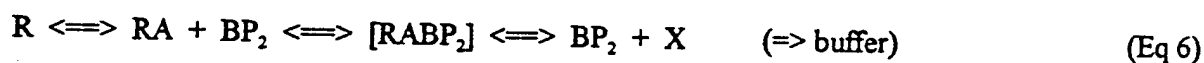
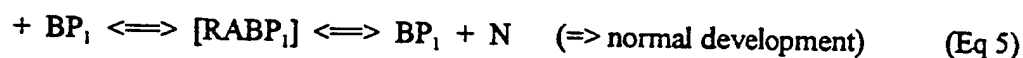
As a step towards determining predictive mechanisms in developmental toxicology, one might propose a theoretical mathematical model and then test the model under various experimental conditions. The developmental sensitivity of an organism to a xenobiotic can be determined by conventional teratology dose-response studies conducted at various times during

gestation. PBPK studies can be conducted at the same gestational times in order to fully characterize the target tissue exposure curves. Combining the data from these two study designs should allow us to characterize the "critical window". A third part of the approach would be to utilize a "developmental movie" generated from the sequential electronic images of the embryos and fetuses that characterized the target tissue development during the critical window; both normal and abnormal sequences would be needed for comparison. Not only would the amount of the teratogen in the target tissue be characterized, but the developmental affect of the teratogen at the time of exposure would also be illustrated. This combined knowledge will allow a description of pathogenesis as a function of target tissue dose; this should provide the molecular biologist with the critical information necessary to design appropriate probes to further investigate site-specific and gestational age-specific mechanisms of teratogenicity.

For our initial investigation, we have chosen retinoic acid and its effects on the developing limb. Too little (vitamin A deficiency) (Wilson et al., 1953) or too much (Kochhar, 1977; Kwasigroch & Kochhar, 1980) retinoic acid will cause malformations in the rat. Retinoic acid produces very specific long bone limb malformations when administered to rats on gestation day 10 and 11 at 100 mg/kg orally (Kochhar, 1977; Kwasigroch & Kochhar, 1980). No limb malformations are obtained if the same dose is administered on either GD 9 or 13. Stress protein synthesis is strongly correlated with exposure of target tissue to retinoic acid, but not with non-target tissue exposure (LaBorde et al., 1995). Retinoic acid is located in the limb bud and is considered a morphogen; it is necessary for normal growth of the limb (Tabin, 1991; Hofmann & Eichele, 1994). Cellular retinoic acid binding protein (CRABP) and retinoic acid receptors

(RARs and RXRs) are located in the limb bud (Tabin, 1991; Hofmann & Eichele, 1994) and mediate the actions of retinoic acid leading to normal or abnormal development. The binding of retinoic acid to the RARs and/or RXRs is thought to be necessary for normal limb development. Important from an experimental perspective is that the limb bud is an easily recognizable tissue in the developing embryo. This knowledge of the role of receptors and the steps in pathogenesis provides a system for correlating expression of specific protein(s) in differentiating cells with a model of normal limb growth during development. The model can then be used to guide experiments to investigate further the molecular events in normal and aberrant development.

As a working hypothesis for the mechanism of action for retinoic acid in the limb bud, the following sequence is proposed:



where R = retinol, RA = retinoic acid, BP_i = the i^{th} binding protein or protein complex, and N, X, and M represent downstream developmental events. We would further propose that the dissociation constant (K_D) for $BP_1 < BP_2 < BP_3$ and the amount of $BP_2 > BP_3 > BP_1$. A mathematical basis for multiple equilibria of the sort proposed (Equations 5-7) has been reported for the binding of thyroxine to thyroxine binding protein, thyroxine binding prealbumin, and serum albumin (Wosilait and Nagy, 1976).

Vitamin A is converted to retinol which is distributed throughout the animal system. At the level of the limb bud, retinol is converted to retinoic acid which binds to binding protein 1 (BP_1); this forms a retinoic acid binding protein complex which in turn is in equilibrium with the regeneration of BP_1 and a product N. N represents the product which initiates a cascade of downstream events resulting in normal development. If there is insufficient RA to bind with BP_1 to form N, then abnormal development occurs. The K_D for BP_1 is the lowest (highest affinity), and the amount of BP_1 is the least so that saturation of BP_1 in Equation 5 occurs prior to any other binding protein being significantly affected. If larger amounts of RA are present, binding to BP_2 in Equation 6 occurs with the product X being formed. X acts like a buffer or sink to take up the excess RA and has no effect on downstream developmental events. BP_2 has a slightly higher K_D with a large amount available for protection against the teratogenic affect of excess RA. If the capacity of BP_2 to bind all of the RA is exceeded, then binding to BP_3 in Equation 7 occurs with the resultant production of M. The downstream effect of the formation of product M is the disruption of normal development and the resultant malformation of the limb.

In this model 'N' is directly tied into alpha of the Gompertz equation (Equation 3 and Figure 5) for normal growth. 'M' disrupts normal growth and increases alpha which lowers the growth curve for the limb. We have developed a visualization of this process by allowing the limb growth to be estimated by a cylinder which is allowed to uniformly "grow" according to the Gompertz equation. We are working on the next step, obtaining computerized images for normal or abnormal growth of the limb during this developmental age.

DISCUSSION

The advantage of a PBPK model for pregnancy is that the potential target organs/tissues of interest are an integral part of the model. However, pregnancy presents additional complexity to the usual PBPK models by adding the dimension of growth to many parameters that would normally be held constant. Although these pregnancy-based PBPK models are complex, they can be readily handled by current desk top personal computers without significant loss of efficiency.

The mathematical model must represent the changes in the growth of the embryo/fetus and the changes which occur in the mother as well as the placenta during pregnancy. The computer model will compute the results of these changes for any day during gestation. The pregnancy model is able to simulate changes resulting from exposure to xenobiotics at any time during gestation and graphically present the consequences of those changes. For example, we can visualize the effects of liver or kidney damage or pathology of the placenta on xenobiotic elimination as well as its distribution in the organs of the mother and fetus throughout pregnancy. In addition, we can model the marked accumulation of fat in the mother and in the fetus in the later stages of gestation which has a large effect on the distribution of lipid soluble xenobiotics. The limitation of collecting sufficient data from humans makes it important to have data on xenobiotic effects from laboratory animals such as rats, mice, and monkeys. Our PBPK model of pregnancy computer program is being modified to take into account species differences, which will expand considerably our ability to understand the effects of xenobiotics during pregnancy.

By being able to easily simulate and visualize the changes in the fate of a xenobiotic during any stage of pregnancy, the toxicologist/teratologist may better understand and probe the interaction of xenobiotics with embryo/fetal target tissue throughout gestation.

The PBPK model's volume and flow constants are not constant during pregnancy. The dramatic changes in both the maternal and fetal systems must be compensated for in the mathematical description of these models. The mathematics of this model (Luecke et al., 1994) were developed to reflect normal maternal and normal and abnormal embryonic/fetal growth during development. After obtaining and applying better estimates of these laboratory animal values to PBPK models of pregnancy, more exact target tissue exposure curves can be obtained. These exposure curves can both increase the precision of extrapolating data from experimental animals to humans which should help improve risk assessments and be used to aid molecular biologists in designing mechanistic probes to aid in the elucidation of mechanisms of action for teratogens. Importantly, the PBPK model constants can be applied to all developmental toxicants, each having its own unique pharmacokinetic parameters.

There is a strong need to make mathematics, visualization, and molecular biology work in concert. All data collectively must be utilized to approach an understanding of teratogenic mechanisms. Such information will be of value in clinical medicine to reduce the incidence of malformations in pregnant women where drug treatment is necessary or in the event of occupational or environmental exposure to chemical agents.

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FIGURE LEGENDS

- Figure 1: Maternal volume (weight) changes across human gestation (modified from data presented in Mattison et al. (1991).
- Figure 2: Growth curve of the human embryo/fetus (modified with permission from Wosilait et al., 1992). The solid line represents the fit to the Gompertz equation (Equation 3) from non-linear regression analysis.
- Figure 3: Human embryonic/fetal liver growth fit to an allometric equation (Equation 4) by regression analysis; insert is same data plotted on ln-ln axis (modified with permission from Luecke et al., 1995).
- Figure 4: Human embryonic/fetal bone growth fit to an allometric equation (Equation 4) by regression analysis; insert is same data plotted on ln-ln axis (modified with permission from Luecke et al., 1995).
- Figure 5: Gompertz curves of growth of human embryo/fetal bones (modified from data presented in Mahony, 1994). The parameters for Equation 3 are listed in the table.
- Figure 6: Growth curve of the mouse embryo/fetus (modified from data presented in Rugh, 1968).
- Figure 7: Laser scanning confocal microscope images; a stacked geometric view of a GD 9 mouse embryo showing the neural folds (NF), heart (H), yolk sac (YS), and somites (SM).
- Figure 8: Laser scanning confocal microscope images; a wireframe view of neural folds from a GD 9 mouse embryo.

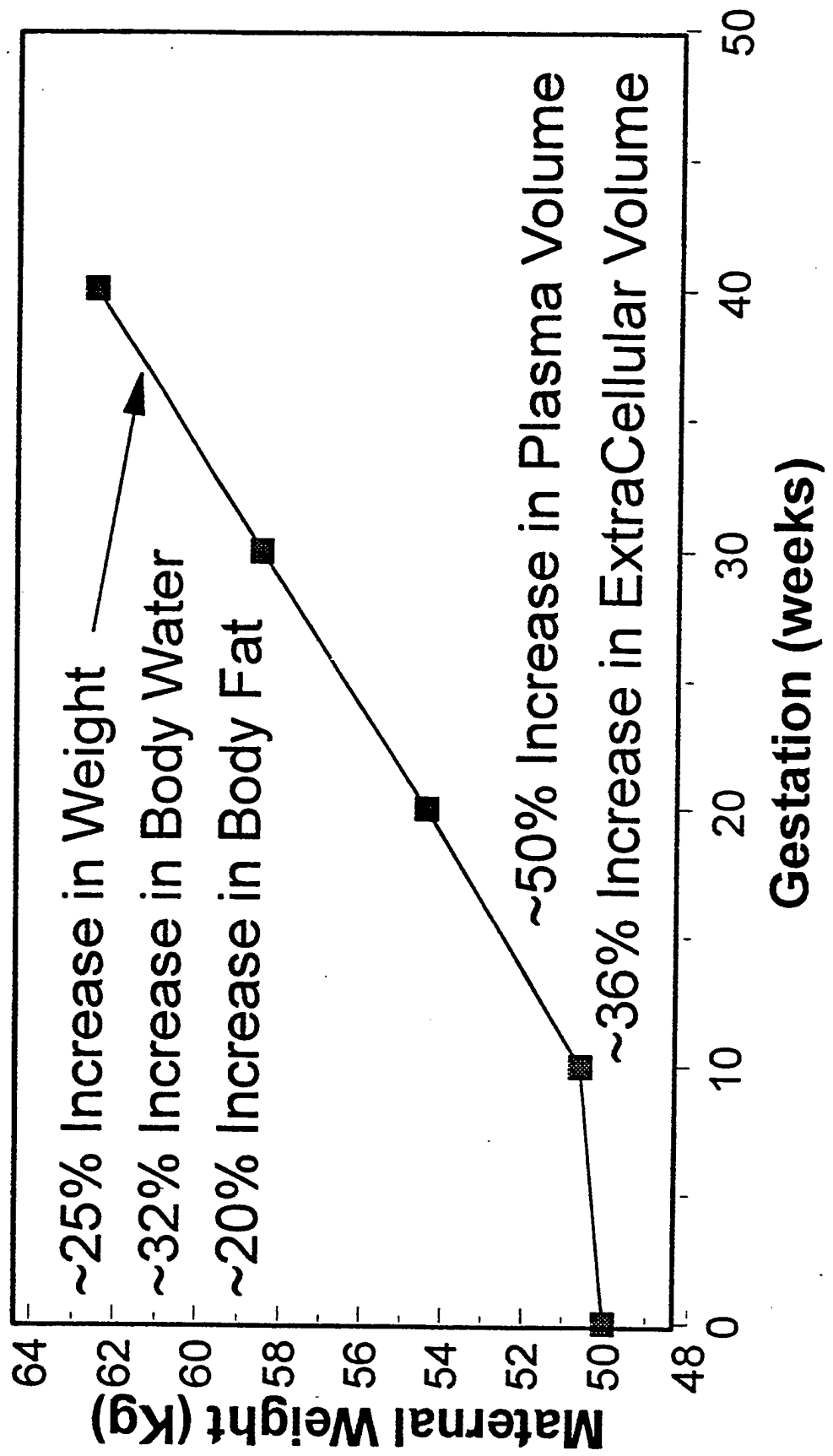
Figure 9: Critical window concept: **critical amount**. For a xenobiotic to be potentially teratogenic, it must expose the embryonic/fetal target tissue to a critical amount of chemical. This over-simplified two compartment model is for illustrative purposes where B represents the amount of xenobiotic in the mother, T represents the amount of xenobiotic at the target tissue in the embryo/fetus, and E represents the amount of xenobiotic eliminated from the mother.

Figure 10: Critical window concept: **critical duration**. For a xenobiotic to be potentially teratogenic, it must expose the embryonic/fetal target tissue to a critical amount of chemical and for a critical duration.

Figure 11: Critical window concept: **critical gestation time**. For a xenobiotic to be teratogenic, it must expose the embryonic/fetal target tissue to a critical amount of chemical, for a critical duration, and at a critical gestation time.

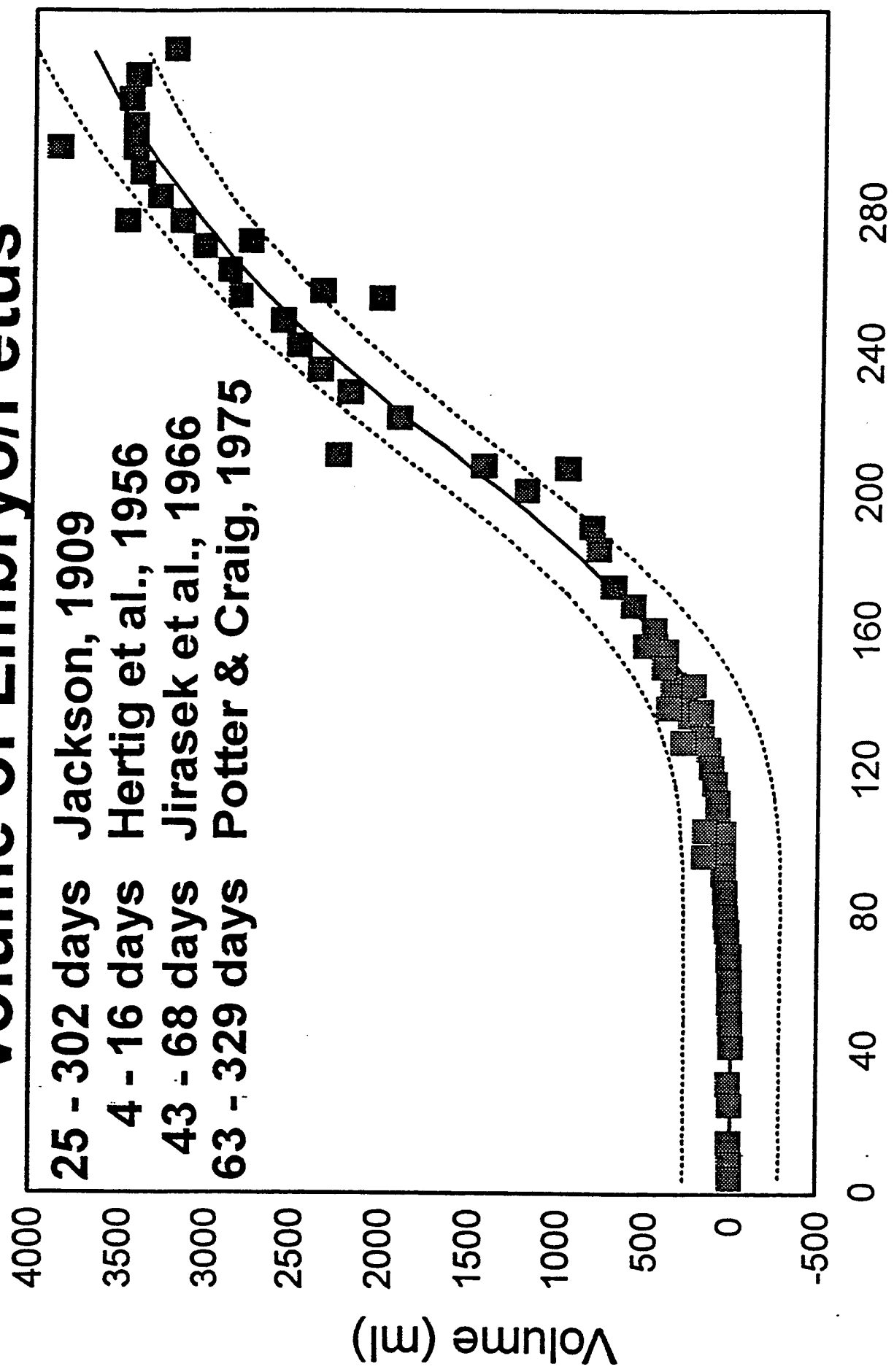
Figure 12: Critical window concept: critical gestation time but with the critical duration outside the critical gestation time. In this example the xenobiotic would not be teratogenic even though the critical amount criteria has been met since the critical duration criteria was not met during the critical gestation time.

Maternal Volume Changes

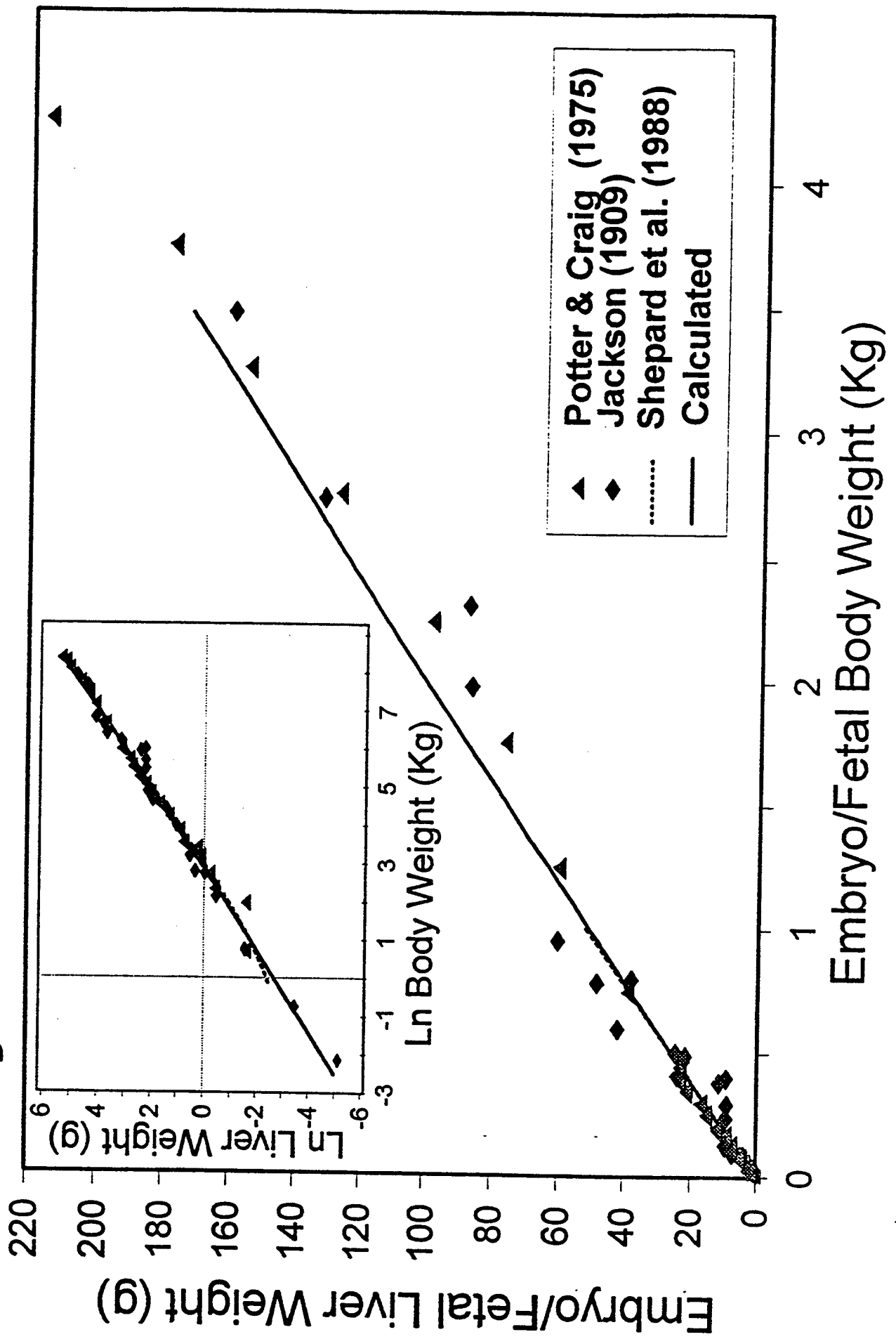


Volume of Embryo/Fetus

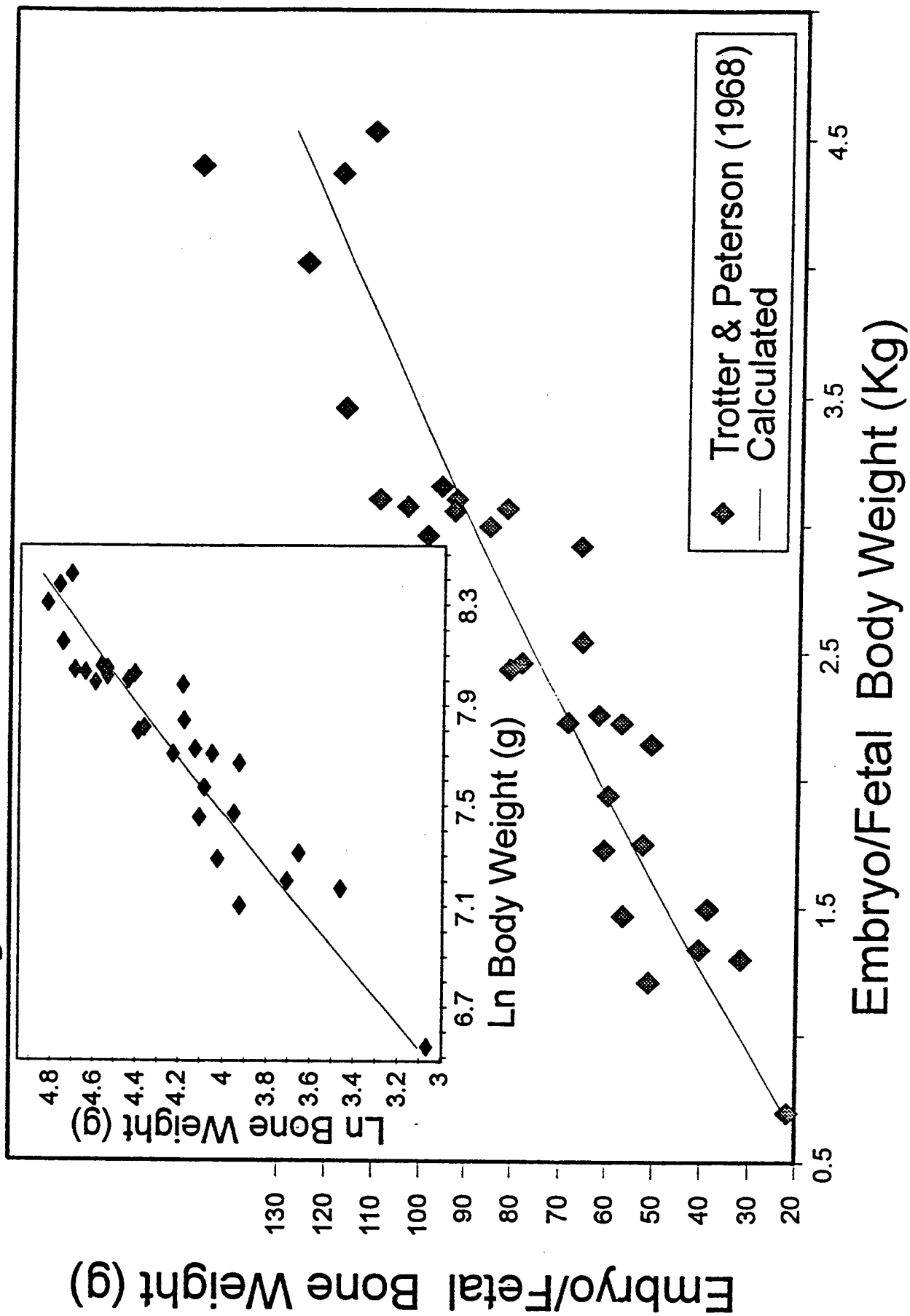
25 - 302 days Jackson, 1909
 4 - 16 days Hertig et al., 1956
 43 - 68 days Jirasek et al., 1966
 63 - 329 days Potter & Craig, 1975



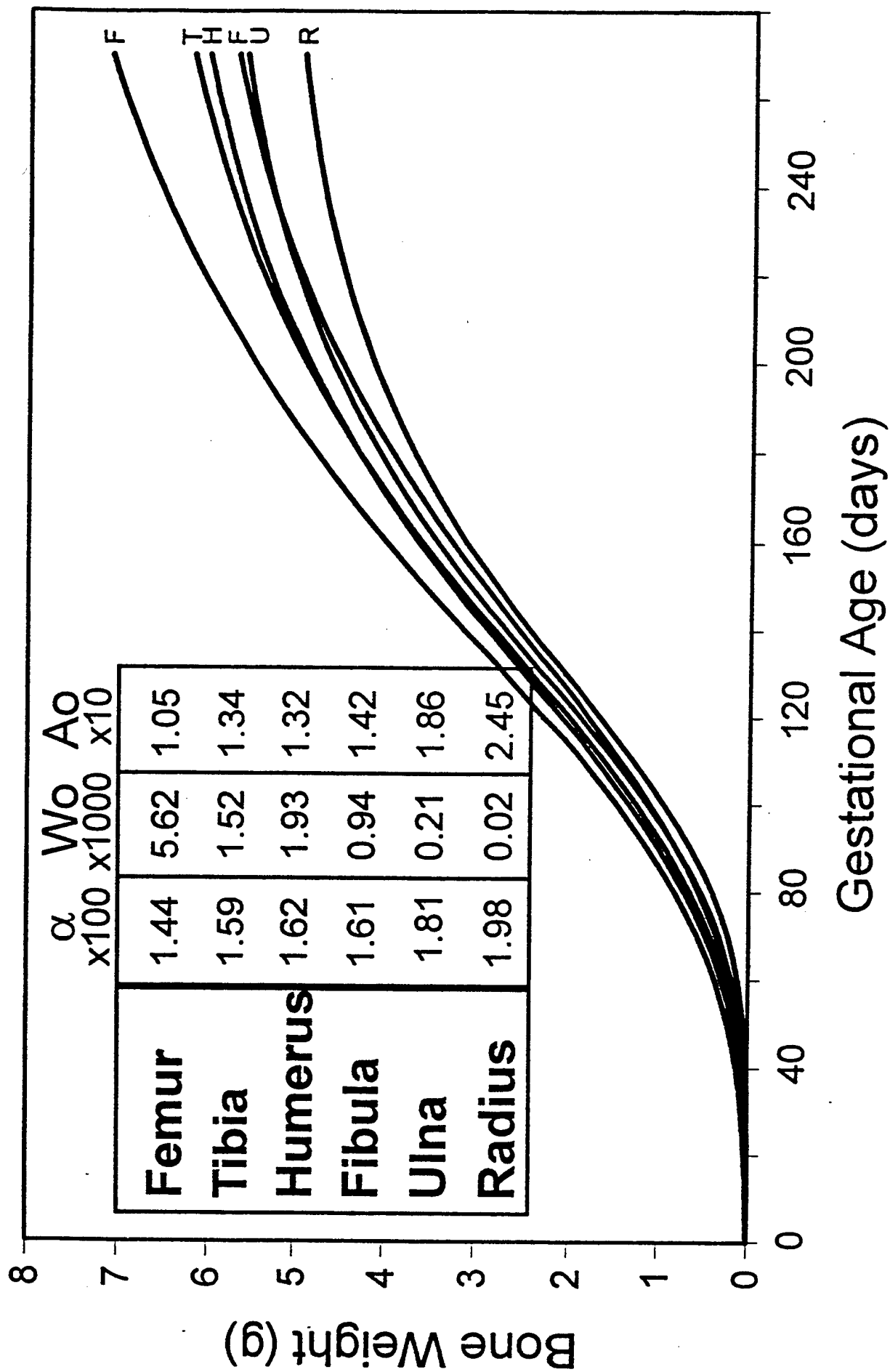
Embryonic/Fetal LIVER Growth



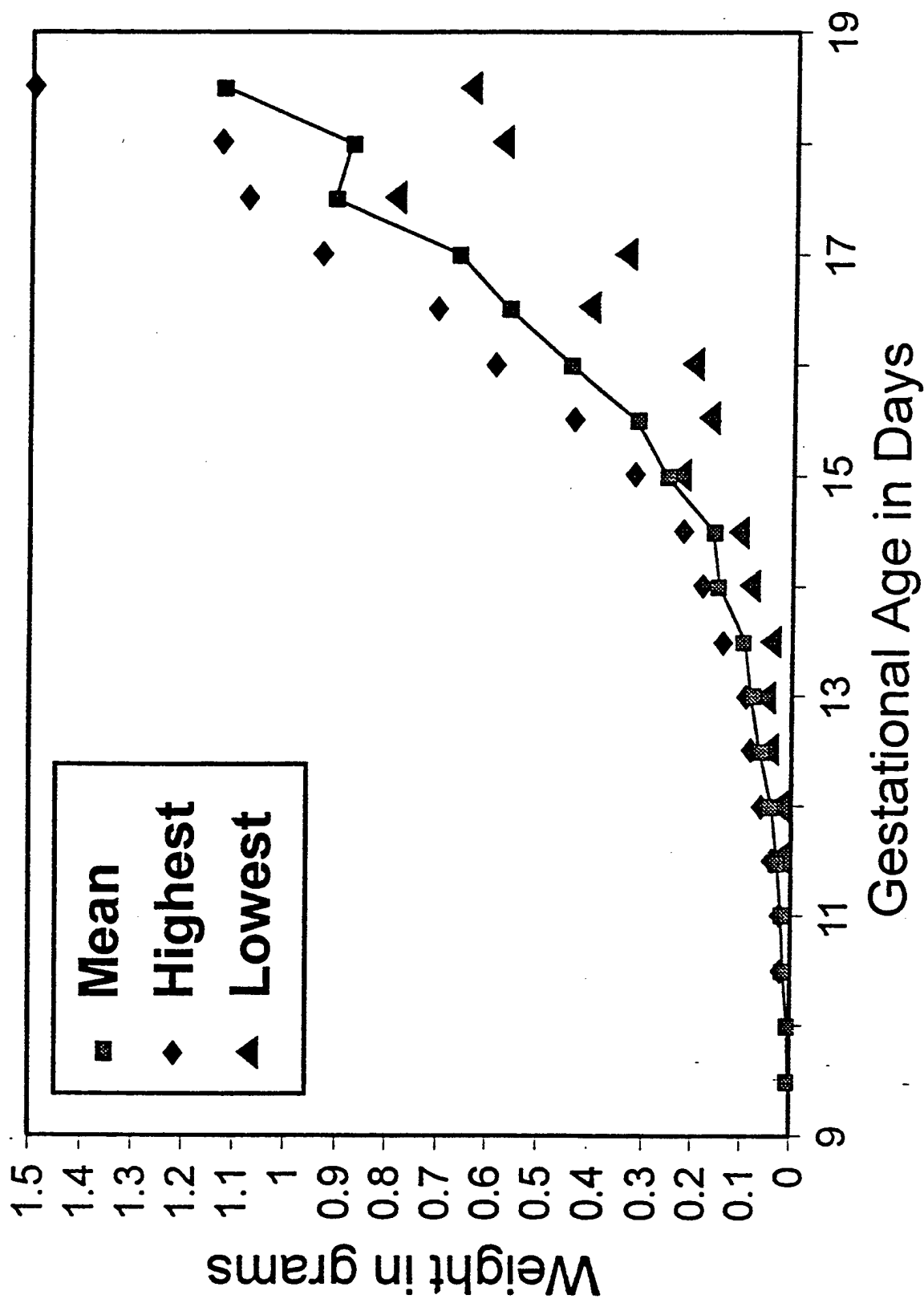
Embryonic/Fetal BONE Growth



Gompertz Equations for Bone Growth

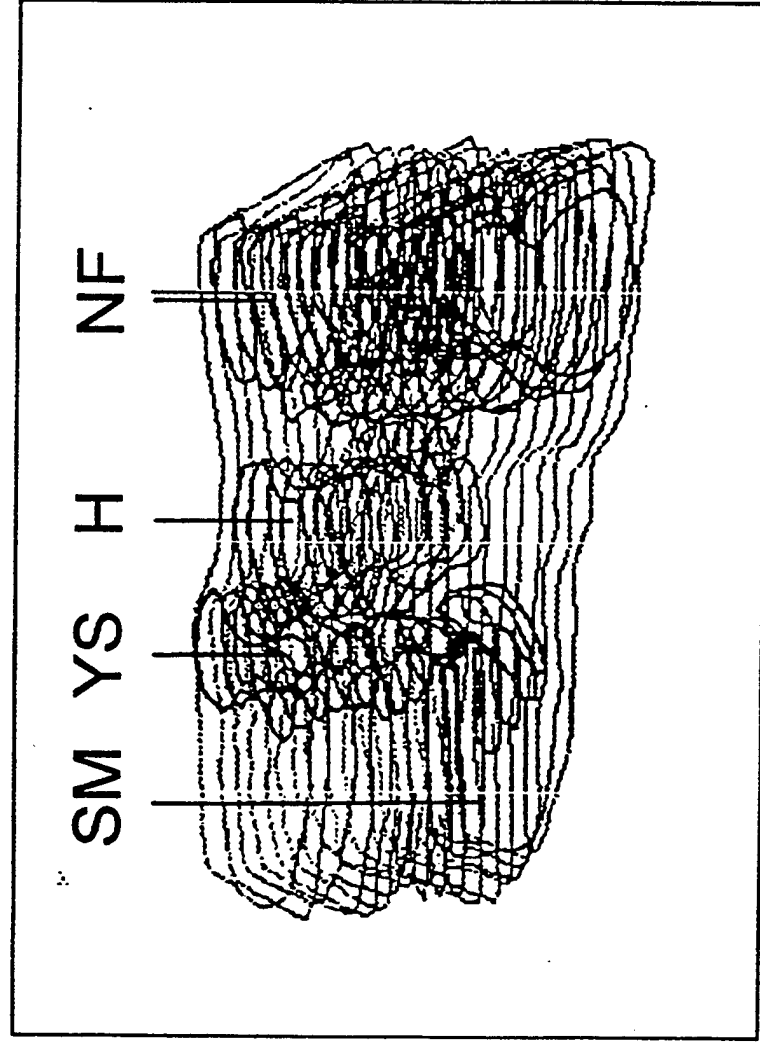


Embryonic & Fetal Growth in the Mouse



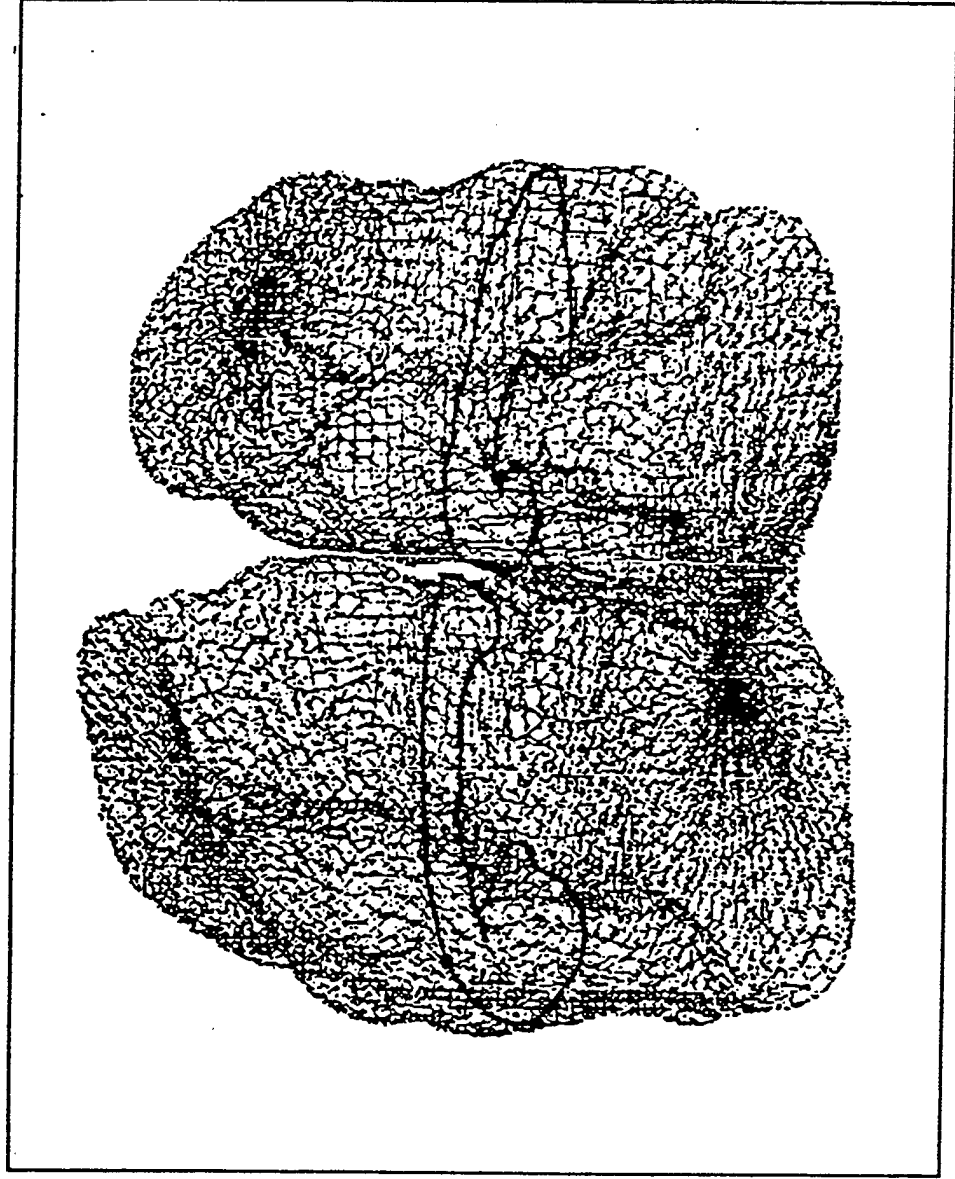
Laser Scanning Confocal Microscope

Stacked Geometric View

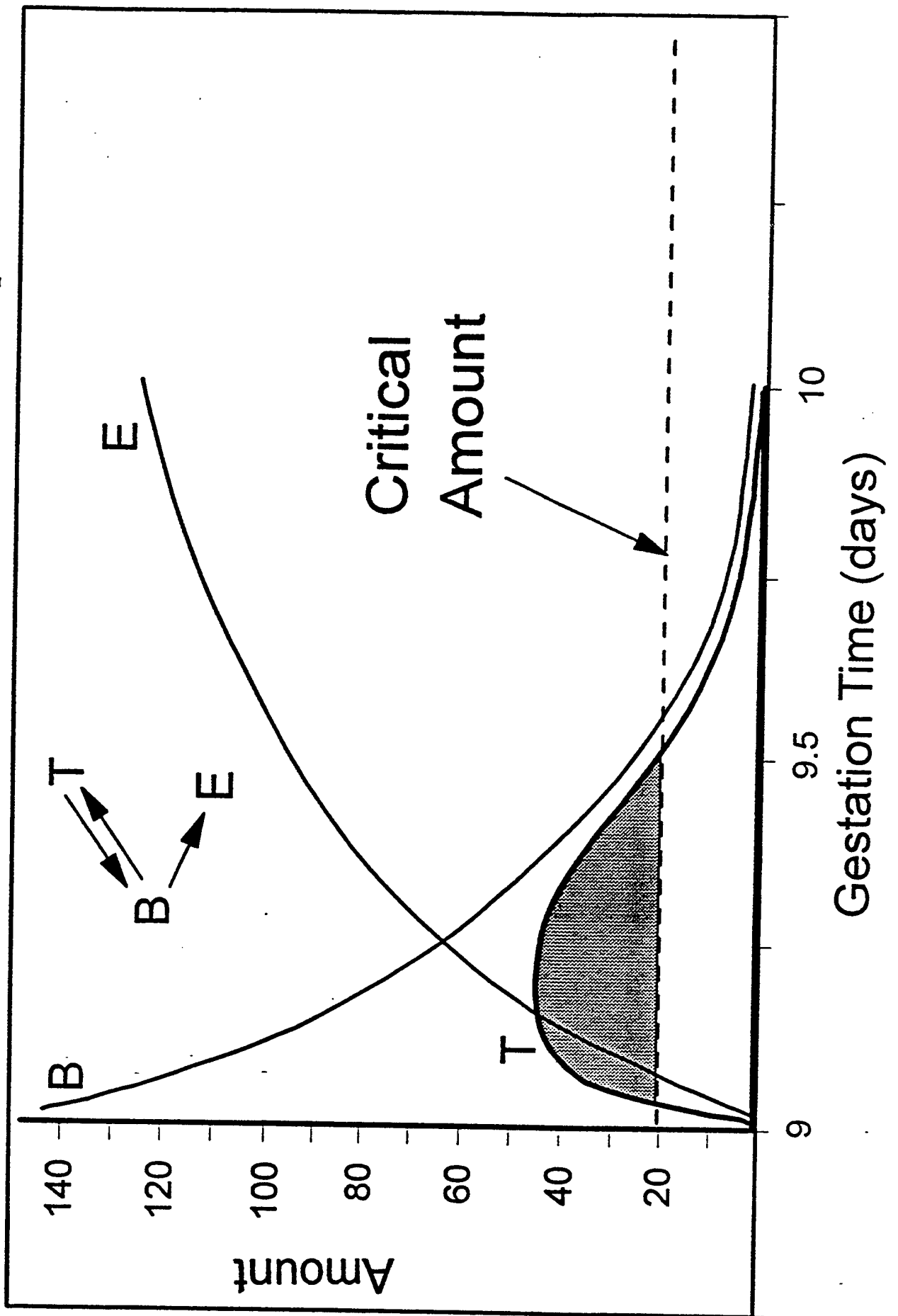


Laser Scanning Confocal Microscope

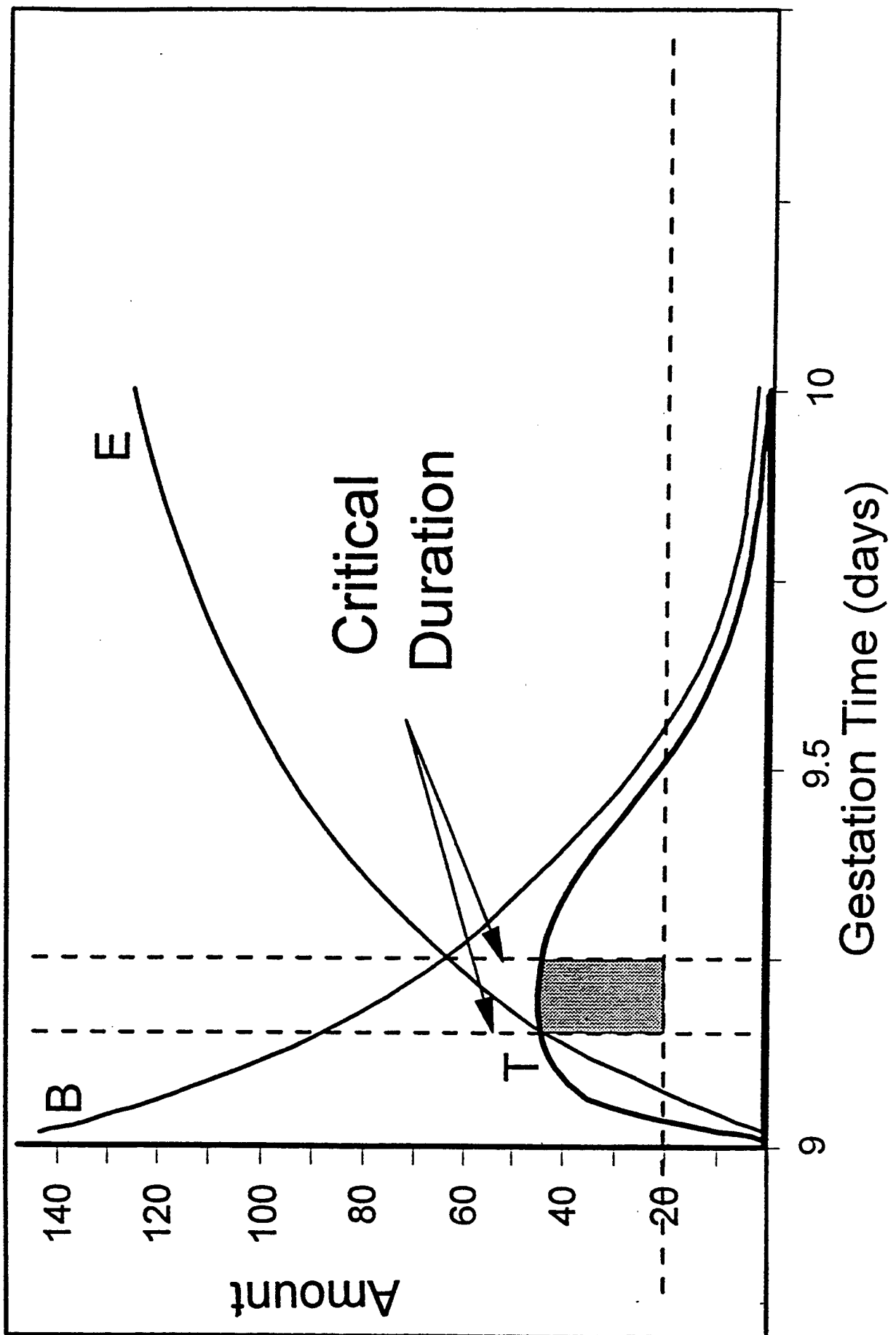
Wireframe View of Neural Folds



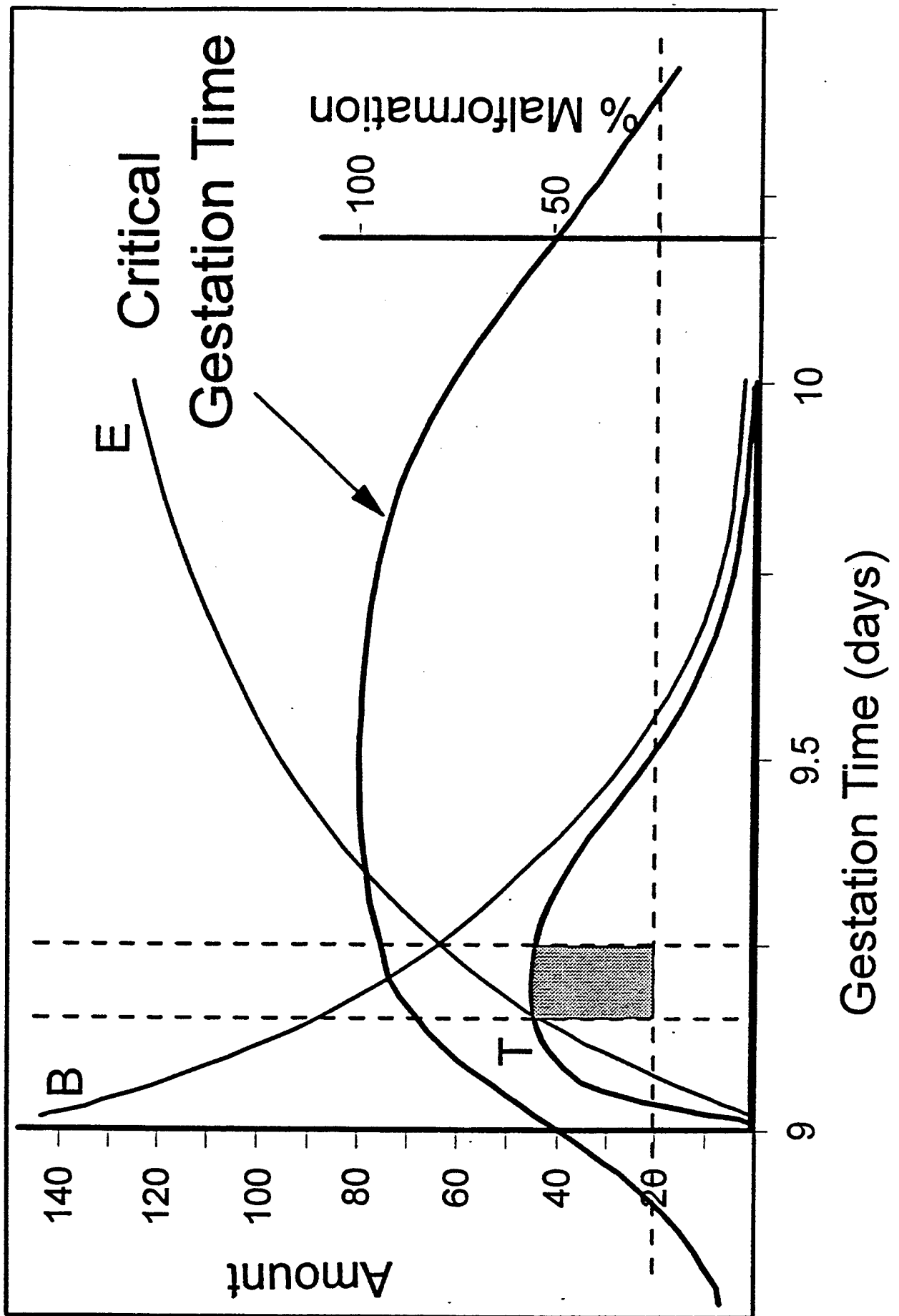
Critical Window Concept



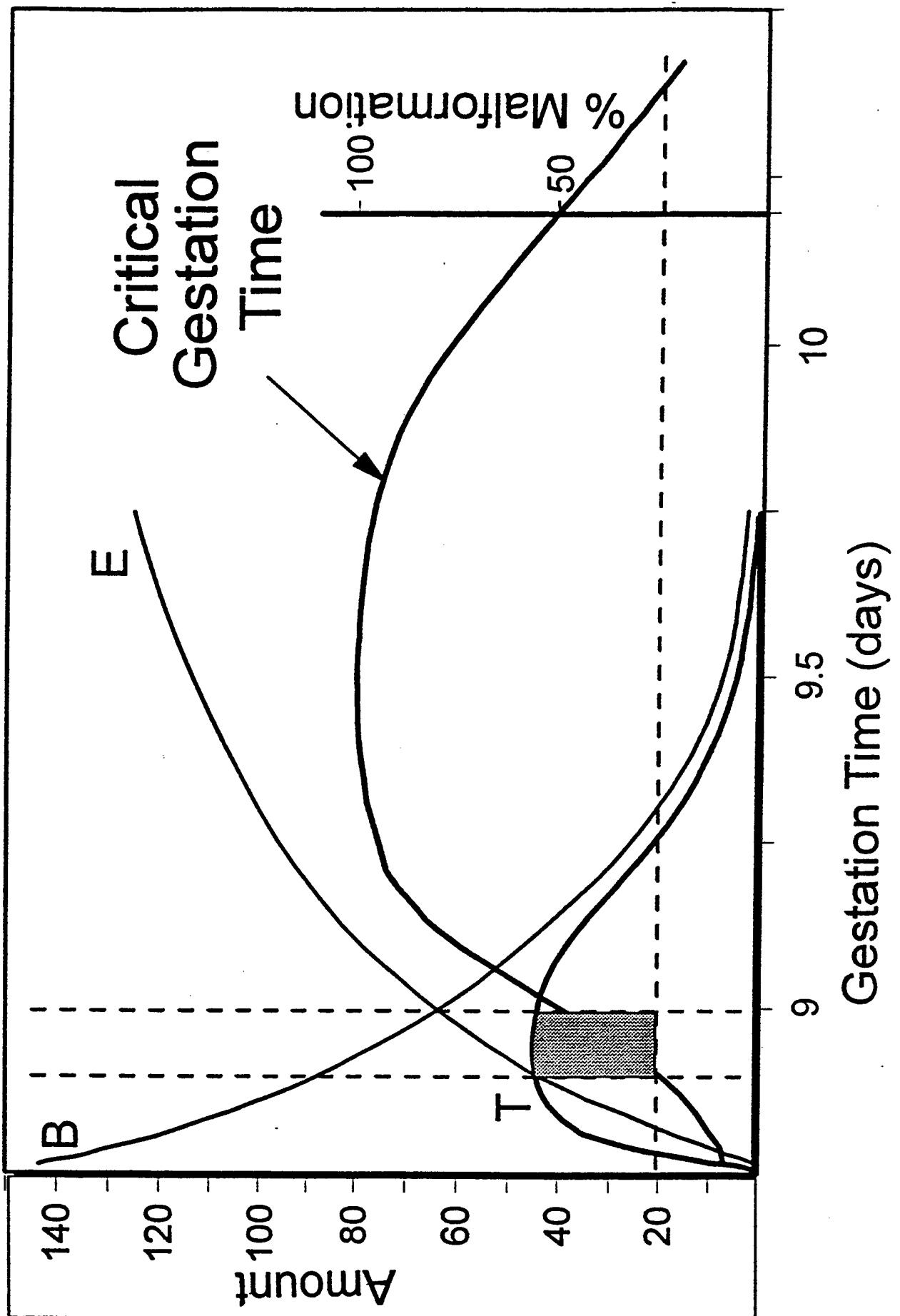
Critical Window Concept



Critical Window Concept



Critical Window Concept



**ABSTRACT OF PRESENTATION:
FILLING DATA GAPS WITH
PHYSIOLOGICALLY BASED
PHARMACOKINETIC MODELS**

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The Agency for Toxic Substances and Disease Registry (ATSDR) is directed by Section 104(i)(5) of the Comprehensive Environmental Response, Compensation, and Liability Act to determine whether adequate information is available to reliably predict the health effects of chemicals such as methylene chloride (dichloromethane; DCM) in human populations. Where such information is not available, ATSDR is required to initiate a program of research designed to supplement existing information. This program may include (but is not necessarily limited to) the following elements (ATSDR, 1992):

- laboratory and other studies to determine short, intermediate, and long-term health effects
- laboratory and other studies to determine organ-specific, site-specific, and system-specific acute and chronic toxicity
- laboratory and other studies to determine the manner in which such substances are metabolized or to otherwise develop an understanding of the biokinetics of such substances
- where there is a possibility of obtaining human data, the collection of such information

In the case of DCM, ATSDR identified several Priority DataNeeds (PDN). Three of these PDNs arose from the fact that the most relevant route of exposure for people living near Superfund sites is ingestion of water containing DCM (oral route), while most of the toxicology studies for DCM

have been conducted by the inhalation exposure route.

Although ATSDR does not currently perform route-to-route extrapolations, the Agency acknowledges that extrapolations from one route of exposure to another may be done on a substance by substance basis after toxicokinetic information has been collected. HSIA and ATSDR recently signed a Memorandum of Understanding initiating a project designed to fill data gaps for DCM with a Physiologically Based Pharmacokinetic (PBPK) model for DCM developed by Andersen et al (1987), and modified by the author for this project.

Three different PDNs have been addressed in this project: (1) acute neurological effects based on prediction of DCM concentrations in brain tissue, (2) subchronic effects on liver tissue related to metabolism of DCM, and (3) reproductive "challenges" to developing fetuses based on distribution and metabolism of DCM in pregnant animals. Results from this project will be presented at the conference.

SESSION IV
RISK-BASED GUIDELINES
(APPLICATIONS OF REFERENCE VALUES)

**ABSTRACT OF PRESENTATION:
NATIONAL CENTER FOR
ENVIRONMENTAL ASSESSMENT:
RESEARCH WITH UNCERTAINTY
FACTORS**

Terry Harvey

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Environmental Protection Agency, Cincinnati, OH

The use of uncertainty factors to modify noncancer, quantitative toxicity values has been in practice for about 10 years. Combining factors via multiplication (1/1,000x) to reduce the levels of toxic concern (and thus clean up) as derived from determinative LAOEL/NAOEL-type data has been characterized as both conservative and of questionable scientific and protective value. NCEA-CIN has been revisiting these cost and public health sensitive issues with a CRADA partner. Methods research is in progress with a view towards improving the technical merit of such calculations. A contemporary treatise on some of these factors is found in a Special Issue of Human and Ecological Risk Assessment, Vol 1, No. 5, Dec. 1995. Improving the accuracy and precision of these factorials can lead to both better public health protection and improved costs for risk reduction decisions.

**ABSTRACT OF PRESENTATION:
ADI, BMD, CEL ... THE ALPHABET SOUP
OF METHODS FOR DOSE-RESPONSE
ASSESSMENT**

M. Dourson

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Humans are exposed to a multitude of potentially hazardous chemicals in their indoor and outdoor air, food, soil, and water. Scientists evaluate the toxicity database for these chemicals to which humans are or may be exposed and attempt to identify and quantify potential risks to health. These risks are then used in conjunction with exposure information for each route (e.g., the daily inhalation rate, the daily consumption of drinking water) to set levels for concentrations of hazardous chemicals in environmental media. These might include the determination of acceptable concentrations in air, food, soil, and water.

The process of human health risk assessment was first described as a four-component paradigm by the National Research Council of the National Academy of Sciences (NAS) in 1983 and was subsequently updated in 1994. The components of this well-known paradigm are hazard identification, dose-response assessment, exposure assessment, and risk characterization. The focus on this presentation is on the element of dose-response assessment, with an emphasis on the methodology used by four agencies: the U.S. EPA, U.S. ATSDR, Health Canada, and the International Programme on Chemical Safety (IPCS). A brief history of the evolution of methods for both cancer and noncancer risk assessment methodologies will be followed by a more detailed discussion of several methods commonly used today, as well as those coming into vogue as the science advances.

Each of the available and developing methodologies is associated with strengths and weaknesses. The decision to use any particular risk assessment method will depend to a large extent on the toxicological data and resources available to the risk assessor. These many methodologies, and the numerous acronyms used to describe them, will be presented as a toolbox from which risk assessors can choose the most appropriate one(s) for any given scenario.

**ABSTRACT OF PRESENTATION:
OVERVIEW OF THE REVISIONS TO THE
EXPOSURE FACTORS HANDBOOK**

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Quantifying exposure or dose can be done using three different approaches: point-of-contact measurement; scenario evaluation; and dose reconstruction. These approaches are described in more detail in the EPA's Guidelines for Exposure Assessment. The focus of this paper is on the type of data necessary to use the scenario evaluation to estimate dose. In the scenario evaluation, the assessor attempts to develop a set of assumptions about how individuals or populations may come in contact with a chemical(s) or other stressor. To calculate this dose, the assessor needs to define a series of exposure factors in an attempt to develop an estimate of the exposure concentration, identify the frequency and duration of exposure, and human body characteristics. The concentration term in the dose equation is exclusively site specific. The data necessary to characterize the other factors generally come from survey statistics, studies of human behavior and characteristics, and the use of models. Statistical data on these factors used in assessing exposure are presented in the Exposure Factors Handbook. This document, first published in 1989, has been very widely used by risk assessors in the Agency and throughout the scientific community. Since 1989, new data have become available on exposure factors, making it necessary to update the Handbook. This paper provides an overview of the revisions to the Handbook, the approaches used to present the data, and the issues and uncertainties with the data.

**THE ROLE OF HEALTH GUIDANCE VALUES IN
PUBLIC HEALTH PRACTICE**

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The Role of Health Guidance Values in Public Health Practice

ABSTRACT

The U.S. Environmental Protection Agency (EPA), the Agency for Toxic Substances and Disease Registry (ATSDR), the Food and Drug Administration (FDA), the World Health Organization (WHO), and other public health and/or regulatory bodies deal on a daily basis with both the derivation and application of health guidance/reference values for toxic chemicals and other substances. Unlike guidance values targeted to workplace exposures in which the intended usage and the exposed population are clearly specified, those derived to address exposure of the general public must be much broader in scope. The potentially exposed populations in the latter situation are more heterogenous, the exposure scenarios more variable and less controlled, and the intended usage/application of those values less rigid than with the workplace exposure limits. As a result, health guidance values such as RfDs, RfCs, MRLs, and ADIs (known collectively to ATSDR as health guidance values, or HGVs) are sometimes inappropriately used. Such misuse of HGVs often results from their being misconstrued as absolute thresholds for the onset of toxicity; that is neither a valid use of HGVs, nor is it their intended usage. The intended usage of these reference/guidance values is for screening or trigger levels that, if exceeded, may warrant further examination of the exposure scenario and the potentially exposed populations. They are intended to be used by public health officials trained in health risk assessment, and only to identify substances of concern at hazardous waste or other sites of environmental contamination. Despite their oversimplification and occasional misinterpretation by well meaning environmentalists and health professionals, these values provide a scientifically sound basis of making a decision on whether further investigation of potential health implications is appropriate.

INTRODUCTION

Human health risk assessment has been the subject of extensive debate, discussion, and review since the publication of the "Redbook" (NRC, 1983). Nevertheless, risk assessment has played a key role in spanning the gulf that is often present between available data and the conclusions provided in a risk characterization. The scientific basis of health risk assessment is grounded in the interpretation of available data and the tools used by risk assessors to extend the biologically plausible range of inference, interpolation, and extrapolation.

Those who work in the environmental risk assessment and human health assessment fields frequently are called upon to render a determination on whether or not a threat to human health is possible in a given exposure scenario. Typically, the basis for determining environmental levels of a potentially toxic or hazardous substance that will be protective of public health are based upon health guidance values (HGVs) developed by federal government agencies. Among the most widely used of these health guidance values for non-cancer endpoints are the Minimal Risk Levels developed by the Agency for Toxic Substances and Disease Registry (ATSDR), the (oral) Reference Doses (RfDs) and (inhalation) Reference Concentrations (RfCs) derived and published by the U.S. Environmental Protection Agency (EPA), and the Acceptable Daily Intakes (ADIs) that have been used by the Food and Drug

Administration (FDA). Despite their wide usage, however, not all who routinely utilize these values are fully aware of what such health guidance values actually mean, how they are derived, and of the many uncertainties inherent in such a process. The goal of this paper, therefore, is to generate a better understanding of what these values actually represent, the multiple uncertainties inherent in their derivation, and the crucial role of biomedical judgment in their application.

WHAT IS A HEALTH GUIDANCE VALUE?

Health guidance values (HGVs) are levels of a chemical or other substance, exposure to which would be expected to cause no adverse health effects over a specified period of exposure. Although there is occasionally apparent conflict among health guidance values developed by different groups, health guidance values in general, particularly for noncancer end points, do have several generic features in common. They represent a level at or below which adverse effects in an exposed population are not anticipated. They generally are duration and route specific, and they represent a sometimes uneasy blend on science and science policy. There are issues where the uncertainties associated with the available data lead us to invoke default assumptions as a matter of science policy.

HGVs are intended for use as screening values (ATSDR, 1992; EPA, 1996, 1989) to identify chemicals of potential health concern at hazardous waste sites. They may also serve to alert primary care physicians to look for symptoms of exposure. They are not intended as precise values above which adverse health effects will occur. Exceedance itself merely indicates that further evaluation may be warranted, although it is likewise recognized that the magnitude and frequency of HGV exceedance is important in evaluation of any potential health risk at a site. The actual precision of the HGV will vary from substance to substance depending upon the scientific data base, and the exposure level of any environmental substance necessary to evoke a toxicological response will also vary from person to person. HGVs should be used only with a full appreciation of the above.

This paper focuses on two types of health guidance values: the MRL, which is analogous to the reference dose; and environmental media evaluation guides, or EMEGs, that are used in our public health assessment efforts at sites in order to categorize these sites with respect to their potential human health hazard. The technical basis for these values is developed and presented in ATSDR's toxicological profiles on substances commonly found at hazardous waste disposal sites (DeRosa, 1994).

The process of toxicological profile development entails a thorough evaluation of the literature, as many documentation efforts do, and

it also entails agency peer review and external peer review, as well as an opportunity for public comment. These documents are developed in a very transparent fashion, and the decisions that are made regarding any internal review comment, public comment, or external peer review comments are part of a legal docket that is available to the public.

ATSDR's health guidance values are used in a hierarchy or tiered fashion as we engage in our evaluation of health issues at sites (Figure 1). This is part of what we at ATSDR do in the context of our toxicological profiles. We look across the toxicological/epidemiological database; we identify toxicity benchmarks; and we apply some uncertainty factors to derive minimal risk levels. We then apply some media specific exposure assumptions to define ATSDR's EMEGs, which serve as screening values for the different environmental media. It should be emphasized here that as we move down this hierarchy from minimal risk levels to environmental media guides and attempt to define the significance of exposure levels, we rely less heavily on default assumptions, and instead rely more heavily on site specific parameters of concern. This is an iterative approach that is consistent with that of the National Academy of Science recommendations (NRC, 1994). This approach is intended to screen against the possibility of false negatives early in this tier, and then to focus resources for further evaluation of substances or issues which we feel actually merit further health evaluation.

There are a number of factors that ATSDR looks at in depth in the health assessment process as it goes beyond the MRL and EMEG and further evaluates potential significance of an exposure at a site. We will look at each of those factors in turn in the ensuing discussion.

DERIVATION OF HEALTH GUIDANCE VALUES

To assist and guide health assessors in evaluating contaminants of concern at hazardous disposal waste sites, ATSDR derives MRLs for oral and inhalation routes of exposure to various chemicals and metals. An MRL is an estimate of daily exposure to a substance that is likely to be without an appreciable risk of adverse health effects, other than cancer, over a specified duration of exposure. MRLs are calculated for three specific durations of exposure: acute (1-14 days), intermediate (15-364 days), and chronic (365 days or longer). These MRLs, their supporting data bases, and an explanation of factors considered in their derivation are included in the respective toxicological profiles. The availability of the internal guidance for the derivation of MRLs by ATSDR was published in the Federal Register (61 FR 25873) on May 23, 1996.

Procedurally, the derivation of an MRL is very much analogous to the derivation of an ADI or RfD. This was an intentional decision by ATSDR in developing its approach to looking at screening values in general. It is a straight forward calculation, based upon the

identification of a toxicity endpoint or a benchmark dose, which is a more rigorous definition of a no observed adverse effect level (NOAEL) or a lowest observed adverse effect level (LOAEL), and some uncertainty factors that are used to reflect the uncertainty associated with the database.

Traditionally, HGVs have been based upon either controlled human clinical studies, human epidemiological studies (usually retrospective), and/or controlled studies involving laboratory animals as human surrogates. From such studies, the lowest dose or exposure level at which an effect considered to be adverse is identified (LOAEL), and the highest level below at or below which no adverse effects have been observed (NOAEL) is likewise determined. Since the population identified in the health study is typically not the same as the potentially exposed population to be protected by the HGV, mathematical adjustments are made to the NOAEL or LOAEL to express the uncertainties inherent in the assumptions and data bases used to calculate the HGV.

The uncertainty factors proposed by Barnes and Dourson (1988) provided the basis for the uncertainty factor approach used by ATSDR (Table 1). Each MRL has its own area of uncertainty surrounding its derivation, and this uncertainty is based primarily upon the characteristics of the study used as the basis of the MRL. The overall (composite) uncertainty factor tends to be a loose upper bound estimate (Dourson, 1993) that accounts for differences

in susceptibility between the test and target species and for sensitivity differences within the human population. This composite uncertainty factor reflects the confidence in the final calculated number and data base supporting that number. The resultant effect of this on the final HGV (MRL) is an estimation of a dose that is likely to be without adverse effects in sensitive individuals for a specified duration of exposure (e.g., chronic in the case of an RfD or chronic MRL). The derivation of the MRL would then be as follows:

$$\text{MRL} = \frac{\text{NOAEL, LOAEL, or BMD}}{\text{UF}}$$

UF

where,

NOAEL = no observed adverse effect level

LOAEL = lowest observed adverse effect level

BMD = benchmark dose*

UF = uncertainty factor

* Benchmark dose is the modeled dose that corresponds to a specified percentage increase in response (Crump, 1995).

EMEGs entail an allocation of an MRL to a particular medium, and represent the coupling of the MRL for a substance with the assumed

intake rate for a unit of environmental medium. For example, in the case of water, the assumed adult intake is two liters per day. This then allows the definition of an EMEG in milligrams per liter (mg/L) that reflects the ingestion rate of two liters per day. Thus, an EMEG for a chemical in water (EMEG_w) would be calculated as follows:

$$\text{EMEG}_w = \frac{\text{MRL} \times \text{BW}}{\text{IR}}$$

where,

EMEG_w = water evaluation guide (mg/L)

MRL = minimal risk level (mg/kg/day)

BW = body weight (kg)

IR = ingestion rate (L/day)

TYPES OF HGVs

The above formula provides a computational method for determining a reference value for a particular substance and for a particular

effect. These values not only provide a route-specific value that would be protective of all potentially exposed populations, but also provide a basis for determining screening or trigger levels for specific exposure media. MRLs and RfDs/RfCs, as well as any similar values such as Acceptable Daily Intakes (ADIs), can be used in specific exposure scenarios to estimate levels of a substance in a particular environmental medium that can serve as a basis to determine whether further evaluation of the health implications of exposure may be warranted. Such screening values are typified by the Environmental Media Evaluation Guides (EMEGs) derived by ATSDR. ATSDR currently has about 220 toxicological profiles under cover, representing some 250 minimal risk levels that are used as the basis for calculating these EMEGs.

EMEGs are media-specific comparison values used in the public health assessment process to identify contaminants of concern at hazardous waste sites. They are calculated for chemicals for which ATSDR has developed Toxicological Profiles, and are derived from MRLs presented in Toxicological Profiles. A corresponding value based upon the EPA reference dose is the RMEG. EMEGs and RMEGs are based upon specific exposure (i.e., ingestion) assumptions which allow translation of an MRL or RfD to an equivalent soil or water concentration. Those assumptions are two liters of drinking water per day for adults (as previously mentioned) and children greater than 10 kg body weight, one liter of drinking water per day for children 10 kg and under, and soil ingestion rates of 100 mg, 200

mg, and 5,000 mg soil per day for adults, children, and pica children, respectively.

EMEGs and RMEGs are based on single-chemical exposure, and do not consider the effects of concurrent exposure to multiple chemicals. They are typically derived for chronic exposures, but may be derived for intermediate and acute exposures as well, since all exposure durations may be appropriate in hazardous waste site exposure scenarios. ATSDR EMEGs are now available on-line through the ATSDR Internet home page:

<http://atsdrl.atsdr.cdc.gov:8080/atsdrhome.html>.

INTENDED USES OF HGVs

In terms of their purpose and intended use, HGVs are used by ATSDR and other groups as screening values. They are not intended to be interpreted as precise values or used as action levels, but instead are to be used as screens that could be indicators of whether further evaluation is warranted in a particular scenario. They can also serve to alert health care providers as to what outcomes they might be concerned about in a particular locality or site vicinity.

Accordingly, ATSDR does not view HGVs as threshold values or values which predict levels of toxicity. They are associated with uncertainty, which needs to be formally articulated; and that is

part of the problem with lists of numbers. Such lists are all too frequently used independently, without due consideration of the narrative weight of evidence which accompanies such values in the context of supporting documentation, such as ATSDR's toxicological profiles.

In the development of public health assessments, ATSDR relies very heavily on three key aspects of information. These are environmental monitoring data, community health concerns, and health outcome data. Environmental monitoring data represent the point at which we use some of the health guidance values that are one product of the risk assessment process. We also fold together with that an in-depth analysis of the toxicity and exposure conclusions in the risk analysis in an attempt to determine what follow-up activities may be needed at a site. Depending on our findings, this could trigger health studies, health education, research, or perhaps an exposure registry (Figure 1).

It should be noted that the risk assessment paradigm has served the environmental health community well in defining the prospective dimensions of an issue from a health perspective. There is also a disease prevention paradigm, which is comprised of surveillance, evaluation, and intervention to control potential exposures and associated toxic effects. This disease prevention paradigm may be used to complement the risk assessment paradigm, and to define

appropriate interventions once the potential for a public health issue has been defined. Appropriate surveillance will allow for the screening of at-risk populations over time, and follow-up health actions can be employed as needed. Such follow-up actions can range from further evaluation or mitigation of exposure to health education of the community, including health care providers (DeRosa and Johnson, 1996).

INTENDED USERS OF HGVs

"CAUTION: PLEASE DO NOT TRY TO USE THIS ALONE AT HOME OR WITHOUT THE ASSISTANCE OF A QUALIFIED HEALTH EXPERT." If that word of caution were placed on each printed copy of an HGV or list of HGVs, perhaps some of the misuse and confusion created by these numbers could be avoided. Such a warning does, however, accurately convey the message that the intended audience for HGVs or comparison values is not the general public. HGVs are intended for the use of qualified environmental and public health officials. HGVs are further intended to be used, not as stand-alone numbers, but in context with the broader scientific knowledge about the substance of concern and in full consideration of the potentially exposed population. Therein lies the primary problem with HGVs: misuse, or perhaps abuse, of a scientifically legitimate reference value by an "unlicensed applicator." Those intended as primary users of the HGVs are provided in Table 2.

FACTORS TO CONSIDER WHEN APPLYING HGVs

A number of factors that must be considered when deciding if and when a particular health guidance value is to be used are provided in Table 3. These factors collectively define the nature of the toxicant(s), the characteristics of the potentially exposed population(s), the nature and likelihood of potential exposures, and the relevance of a particular health guidance value to a given site scenario.

Characteristics of the Exposed Population

The first of the factors to be considered is the nature and composition of the potentially exposed population. Characteristics of the exposed population, including host factors such as age, gender, health, and nutritional status, are then coupled with some site specific concerns, such as likelihood and frequency of exposure, as well as any concurrent exposures to other chemicals from other source.

Nature of the Potential Exposure

The nature of the exposure, intricately related to the nature of the toxicant itself, is something that must be looked at in terms of the chemical and physical properties of the toxicant as it would

relate to the environmental partitioning of the material as it is released from a site. We look at the potential routes of exposure based on that environmental partitioning, and try to assess the likelihood of concurrent exposures. We also look at the potential for joint toxic action of multiple chemicals at a site, focusing on mechanism of toxic action of the individual chemicals as the means of providing insight into the potential for joint toxic effects.

In addition to the nature of the toxicant in question, the route(s) of exposure, the history of past exposures, the level of any known or potential current exposures, and the possibility of additional/future exposures, as well as any concurrent exposures to other substances, must all be taken into consideration when making a determination of how to apply a particular HGV. While HGVs are substance- and duration-specific, that does not necessarily overly restrict their usage as input to the judgmental processes of health assessors in determining the overall health risk to specific exposed populations.

The Duration of Exposure in a Given Scenario

Critical evaluation of the appropriateness of any guidance value for a particular exposure scenario is a crucial step in the assessment of the significance of human exposures at any site. In ascertaining the duration of exposure against which an HGV is to be

compared, the likelihood of past exposures must be considered along with current and potential future exposures. For instance, if a residential or other potable water well is found to be contaminated, the health assessor must take into consideration the length of time that an individual or individuals have already been using the water for drinking purposes, in addition to any projected future exposures.

To underscore the importance of not taking the HGV as a stand-alone value, an MRL might not necessarily be appropriate for all exposure situations. In evaluating the applicability of an HGV for a given situation, the health risk assessor must ask whether the duration of the actual critical study exposure was similar to, or different than, the potentially exposed population at a waste site or other scenario. That is, the nature of exposure in the critical study (continuous inhalation, 6 hr/day inhalation, number of days exposed per week, etc.) would have to be evaluated in the context of the exposed human population.

For example, it might be the case that a particular HGV was based upon intermittent or part-day exposures, such as when the test animal was exposed by inhalation for 6 hours per day, 5 or 6 days per week, and that those exposures were adjusted to account for an HGV based upon 24 hour a day, 7 day per week exposure. In a given site-specific scenario, however, the duration adjustment applied in calculating the HGV might not be necessary for the exposed human

population. The bottom line is that all factors impacting upon the calculation of an HGV and its relevance in any human exposure scenario must be carefully weighed by trained professionals as part of the biomedical judgment process.

Scientific Basis of Health Guidance Value

The scientific basis of the health guidance value is, of course, pivotal to any credible health risk assessment. Since MRLs are calculated in such a fashion as to be protective of the most sensitive subgroups of the human population, it may be the case that a particular MRL or the toxic effect upon which it is based may not be fully relevant to the exposed population and might be inappropriately restrictive in a given exposure scenario.

The age, gender composition, overall health, and nutritional status of the potentially exposed population must be considered along with other factors (Table 3) when ascertaining the applicability of any specific HGV. Many effects of toxic substances present a particular health threat to the very young, the very aged, and pregnant or lactating women. Those are also groups with nutritional needs specific to their stage in life. Thus, chemicals or other substances that have the potential to affect either neurological or structural development (e.g., lead) or the absorption of essential nutrients or medication (e.g., certain metals), or the metabolism and efficacy of life-sustaining pharmacotherapeutical agents may

present an increased risk to those populations and must be taken into account when evaluating potential health risk posed by environmental contaminants.

The critical effect/endpoint used as the basis for HGV calculation must be relevant to the potentially exposed human population. For example, the threat of methemoglobinemia from nitrates and nitrites in very high levels (parts per thousand) in drinking water may present a real threat to pregnant women and newborns in the first three months of life, but would be essentially harmless to persons outside of those particular high risk groups. Similarly, if the critical effect in an HGV study was based upon a route of administration different than one that might be encountered by the potentially exposed human population living near a hazardous waste disposal site, (i.e., gavage vs. soil ingestion), the HGV would have to be considered in light of the probable exposure scenario and relevant factors such as bioavailability. In the derivation of an HGV, adjustments are typically made to provide the most conservative approach, which may make a MRL too stringent in some exposure scenarios. All of these factors have a role in determining the applicability of the bare HGV in a particular exposure scenario; and they all must be taken into consideration when making a judgment on whether a health threat exists, the extent of any threat, and for which population or individuals the potential health threat is the greatest or most likely.

Once more, it should be stressed that a health guidance value is something that we view as a level that does not present a significant health concern. HGVs likewise do not represent threshold values, nor are they intended to be action levels. And any HGV must be considered in light of the exposed population and the overall exposure scenario. In the case of our health guidance values, we attempt to look at all the factors that are listed in Table 4. These factors are not novel to any evaluation of issues associated with exposure to chemicals, but rather are the things that we attempt to formally address as we look at a site in the context of our toxicological profile for a particular chemical.

Background of Substance in Environment

Finally, we take into account the background level of the substance in the environment. In cooperation with EPA, ATSDR has published a list of 275 priority pollutants based on their frequency of occurrence at waste sites, their toxicity, and the potential for human exposure (Federal Register, 1994). We have collaborated with the laboratory at the National Center for Environmental Health at CDC to develop an array of analytic protocols that can be used to define more accurately these background levels of exposure in the environment both in contaminated media and in human tissues and fluids.

Environmental background level is an area that is occasionally

overlooked by some, but nonetheless an area of considerable relevance. Before making a determination of whether a particular action is necessary to protect the health of potentially exposed individuals, it is always necessary to ascertain the local background level (if any) of the substance in question. Background levels should also be obtained for the state and the U.S. as a whole for comparison purposes. If the normal background level of a particular substance in a geographical area exceeds the HGV, strict adherence to the HGV might be totally inappropriate. For example, the background concentrations of certain metals, such as arsenic and selenium, may be higher in the soil than a chronic oral EMEG_s or RMEG_s for those elements. This is not necessarily problematic to the health assessor at ATSDR, however, since he or she is aware that HGVs do not fully reflect such factors as bioavailability, and since those HGVs are intended as only screening/trigger levels to indicate that further evaluation of the exposure scenario might be warranted in order to properly assess the potential for human health concern. This underscores the necessity of skilled biomedical judgment in the site-specific evaluation process.

The health assessor must also determine whether there is a regulatory value governing air or water levels of the substance. (e.g., NAAQS, MCL, etc.). Consideration must also be given to those substances known to be essential for normal physiologic function (essential nutrients).

LIMITATIONS OF HGVs

The user of an HGV should understand what that value is, but just as important in ensuring the proper use of HGVs is an understanding of what they are not. Perhaps rule or instruction number 1 to potential users of HGVs should be that they do not represent threshold levels for toxicity. It should be noted that the dosage or treatment levels used in MRL and RfD/RfC derivation are appropriately called "no observed" effect levels, and not "no observable" effect levels, as they are descriptive only of the dosing conditions in a specific scientific study, and are not meant to imply a threshold level for a specific effect.

It should also be noted that the NOAEL focuses only on the dose representing the NOAEL and does not incorporate information on the slope of the dose-response curve at higher doses at which effects are observed. Further, the spacing of the doses in the critical study influences the level identified as the NOAEL. Consequently, a wide dose spacing may result in a NOAEL that considerably underestimates the actual threshold for an adverse non-neoplastic effect. A second limitation of the NOAEL approach is that an identification of a NOAEL is based upon a statistical test of the null hypothesis that the response rate at a particular dose is equal to the response rate of a control group (Dourson, 1993). As the sample size is increased, the test becomes more sensitive, making it more likely that the null hypothesis will be rejected.

This tends to push the NOAEL toward a lower value as the size of the sample population is increased. The converse is also true --- that with limited sample sizes, studies are less sensitive and the affects may not be detected, resulting in acceptance of the null hypothesis (i.e., a false negative).

These observations serve to illustrate that the NOAEL/LOAEL approach to human health risk guidance value derivation does not, and is not intended to, provide an absolute prediction of a toxicity threshold above which toxic effects are likely to occur. On the contrary, an HGV merely represents a level at or below which adverse effects are unlikely to occur during or following a specified period of exposure. There is no inherent presumption or implication that occasional excursions above the HGV will necessarily lead to a manifestation of toxicity, although the risk of experiencing adverse health effects will be expected to increase with increasing frequency and magnitude of excursions above that level.

Alternate approaches to the traditional NOAEL/LOAEL method, such as the benchmark dose approach (Crump, 1984) which allows selection of a specific (e.g., 1%, 5%, 10%) percent response level within the experimental dose range, are being employed in health risk assessment with increasing frequency. Such approaches have advantages over the traditional approach, but typically have requirements for larger and better data bases. These approaches

are actually extensions of the NOAEL/LOAEL approach with a little more flexibility for the application of biomedical judgment at multiple steps in the process, rather than just in effect selection and estimation of uncertainty. In reality, it may be that there is no "one size fits all" solution to methodological approach to health risk assessment, just as the calculated health guidance value like-wise represents no "one size fits all" number.

In conclusion, it should be borne in mind that health guidance values have been developed across a number of different agencies and by a number of different countries. Each may be numerically different, but since each has its own realm of application, one size does not necessarily fit all. It is therefore incumbent upon all health risk assessors to think in terms of harmonization whenever possible and practicable, and to be able to explain how these different values do interrelate in order to credibly explain our assessments and conclusions about any perceived health threat to the public.

SUMMARY

Health Guidance values are numbers representing known or estimated safe levels of exposure, which are intended to serve as trigger levels to suggest that further evaluation of a site may be warranted. They are not precise values, nor are they intended to

be considered as threshold or other safe health limits. They are not intended for use of the general population, but rather by public health officials trained in the sciences of medicine, toxicology, and/or environmental health risk assessment. HGVs should be used in the full context of the potentially exposed population and all reasonable possible exposure scenarios. While new and alternative methods of estimating potential health risk are continually being investigated and employed where appropriate, the resultant HGV is expected to remain merely a screening tool for the judgmental use of environmental health professionals.

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TABLE 1

UNCERTAINTY FACTORS EMPLOYED BY ATSDR
IN THE DERIVATION OF MRLs^{a,b}

<u>Uncertainty factor</u>	<u>Used to Account for Uncertainty in</u>
3 - 10 ^c	Extrapolation from results observed in laboratory animals to humans (INTERSPECIES DIFFERENCES)
1 - 10	Differences in sensitivity among humans (INTRASPECIES VARIABILITY)
3 - 10	Use of an LOAEL for MRL derivation
3 - 10	Lack of completeness of data base

^a Maximum uncertainty factor in MRL derivation is limited to 3,000 by science policy

^b ATSDR, unlike EPA, does not extrapolate from one study duration to an exposure of a different duration (e.g., from intermediate/subchronic to chronic)

^c Usually 10, but may be 3 in case of relevant studies in primates; typically 3 in the case of inhalation MRLs in which EPA dosimetry adjustments (EPA, 1989) can be applied.

TABLE 2

INTENDED USERS OF HEALTH GUIDANCE VALUES

1. U.S. Government scientists, medical practitioners, and public health officials
2. State, county, and city environmental and public health departments
3. Fire departments and other local or state emergency management offices
4. Private sector organizations involved in mitigation activities at hazardous waste sites

Unintended, But Nonetheless Occasional, Users Include

1. Environmental advocacy groups
2. News media
3. Citizens (including attorneys involved in litigation)

TABLE 3

CHARACTERISTICS OF EXPOSED POPULATION

Age

Gender

General health

Nutritional Status

Likelihood/Frequency of Exposure

Concurrent Exposures

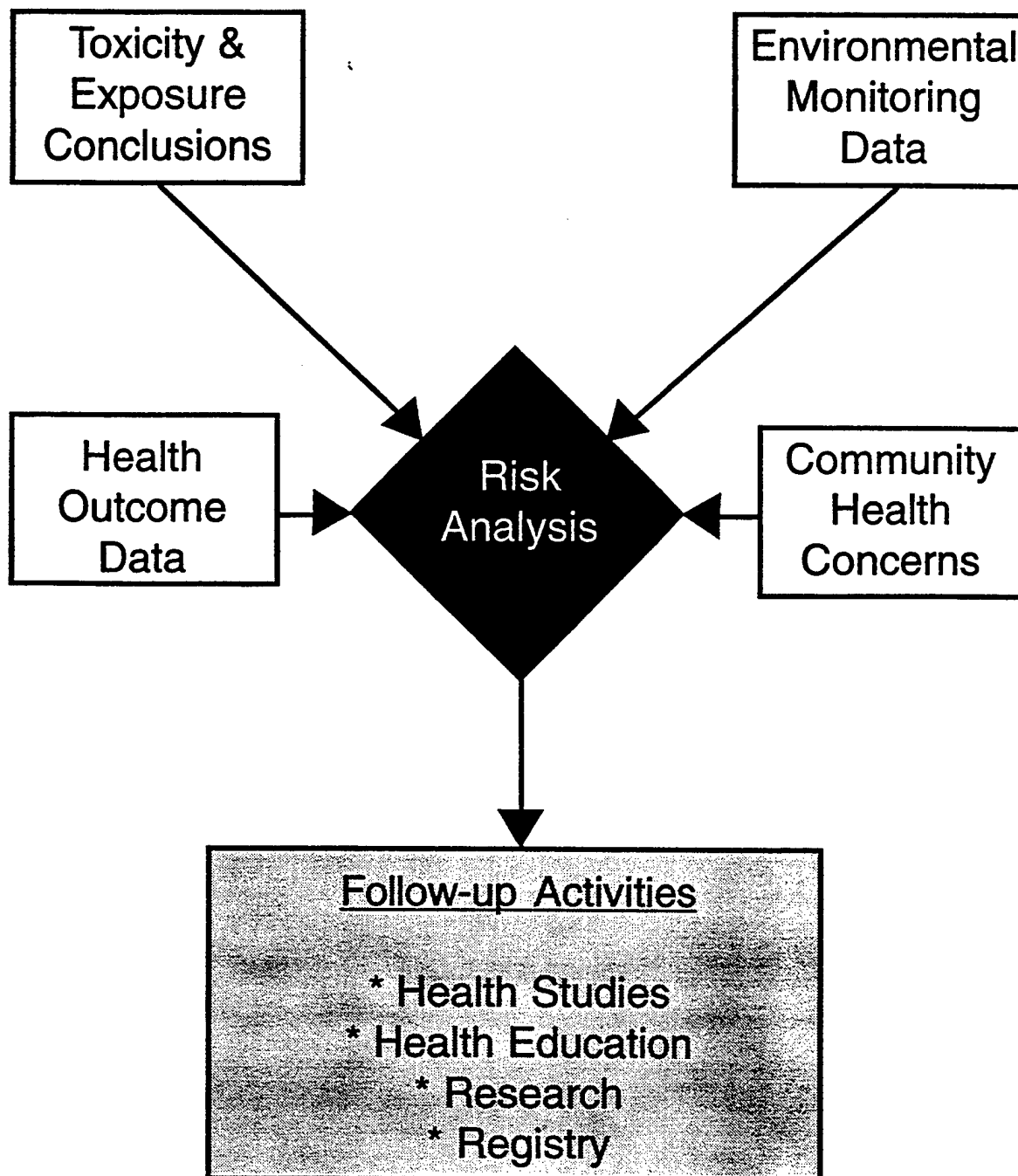
TABLE 4

FACTORS TO CONSIDER WHEN APPLYING HGVs

1. Characteristics of exposed population
2. Nature of Exposure
3. Duration of Exposure(s)
4. Scientific basis of health guidance value
5. Background level of substance in environment

Figure 1

Determination of Significant Human Exposure



**ABSTRACT OF PRESENTATION:
RECENT ADVANCES IN QUANTITATIVE
NONCANCER RISK ASSESSMENT
METHODS: TOXICOLOGICAL AND
MECHANISTIC CONSIDERATIONS**

Elaine M. Faustman

Department of Environmental Health, School of Public
Health and Community Medicine, University of Washington,
Seattle, WA

This talk will focus on two areas of research for quantitative risk assessment methodologies for noncancer endpoints. First, the results from our multiyear analysis of benchmark methodologies for developmental toxicity will be presented. A discussion of generic versus endpoint specific models will also be included. These models will be compared with traditional NOAEL approaches. Applications of benchmark methodologies for both quantal as well as continuous endpoints will be discussed. The second part of this talk will discuss the feasibility of using biologically based dose-response models for evaluation of noncancer endpoints. The biological information that is incorporated into these models includes timing and rates of dynamic cell processes such as differentiation and migration as well as growth and replication. Heterogeneity of cell kinetic rates across populations of individuals is explicitly modeled. A flexible class of dose-response models is developed, including threshold and non-threshold curves with the aim of explaining the pattern of malformations rates as a function of both dose and time of exposure. Methylmercury will be used as an illustration due to the availability of studies evaluating this agent's effects in multiple species and in both *in vivo* and *in vitro* evaluations. The general applicability of these cell kinetic models for evaluating noncancer endpoints will be discussed.

**ABSTRACT OF PRESENTATION:
SESSION SYNOPSIS**

Dale Hattis
Clark University, Worcester, MA

Is there anything new under the sun in noncancer risk assessment? The 100-year anniversary of the first publication of the 100-fold safety factor approach is rapidly approaching in the year 2054. By then, will we have different safety/uncertainty factors for different kinds of adverse effects and different kinds of agents? Will the modest incremental reform of the "Benchmark Dose" methodology be followed up by a more comprehensive reconstruction of the process? Will anyone be able to say how big is the risk that is considered "acceptable" by those who derive "Acceptable Daily Intakes"? Will we be able to provide at least some quantitative estimates of health risks above and below the reference dose? Will we have a substantial enough database of empirical information on the accuracy of different interspecies projection formulae, and the extent of human interindividual variability in susceptibility, to reconsider the generic 10-fold values used as safety factors? This session will reveal whether these questions are on the radar screen of current leaders in this field.

SESSION V
RISK COMMUNICATION IN
THE FEDERAL GOVERNMENT

ENVIRONMENTAL RISK COMMUNICATION

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You're the proclaimed expert on the topic of toxic effects of a particular chemical that your organization has been using for some time now. The question has been raised in the community as to whether or not there has been a release of that chemical into the community's environment causing adverse health effects. The members of the community want answers... NOW.

Since you're the resident expert, your department chair, division chief, commander, boss, has volunteered you to be the organization's spokesperson at a public meeting. The meeting is scheduled to be held a week from now. You're expected to address the presence or absence of the chemical in the community. You're also to address health effects or lack of health effects of that chemical, and safety measures being taken by the organization to ensure the public's health and safety.

Oh, by the way, did I mention the meeting attendance is expected to exceed the capacity of the auditorium it will be in? The media will be there looking for a hot story and activist members of at least two environmental groups are expected to attend with banners and posters proclaiming the illnesses and deaths you're causing.

What are you going to do? Hopefully, you've become familiar with communication techniques that focus specifically on communicating effectively in such a hostile, low trust/high concern situation. This is what environmental risk communication is about.

The foundations of Environmental Risk Communication come from academic research. It is based on a combination of sources; including surveys, case studies, and field testing and experimental testing of messages and messengers. The skills advocated in Environmental Risk Communication literature and education have been shown to be effective both in theory and in practice. Much of the current literature on Risk Communication is drawn from observations made in public meetings and public availability sessions conducted by government and industry groups in working with the public and the media.

Given Environmental Risk Communication skills focus on that specialized situation of low trust and/or high concern; one asks the question "just why is effective communication so difficult when dealing with environmental concerns?"

Communication difficulty stems from a variety of sources. Among the most frequently cited is the complexity of environmental issues; especially when there is a perception of contamination, adverse health effects, and/or clean up activities that seem to drag on and on.

Additionally, especially with respect to federal government activities, there is a general sense of low trust and credibility in the public's view. Often times information that the public receives from federal government spokespeople and agencies is misleading or perceived to be misleading. Finally, frequently the public has perceptions, or misperceptions, about both environmental concerns and the credibility of government representatives.

With effective communication so difficult, the next question that is frequently asked is "so why communicate?" Depending on what the motivation is, there are several reasons to communicate on environmental issues.

First, there is a current trend in the government to be more open with the public on environmental issues. One of the driving forces behind this is current environmental law that directs communication with the public. Known as community right-to-know laws, communication is frequently approached from the standpoint of a legislated requirement. Partly in response to the laws and partly in response to the perception of the public that the government and industry have hidden information and covered up in the name of national defense, organizations must now open up and make an honest

effort to communicate with the public about contamination that may have occurred over the years. As progress is made in partnering with communities, we are seeing more and more effective communication resulting in improved community support.

To better understand the need for specialized communication skills, we need to first understand the difference between "normal" communication situations and communication situations in which an air of low trust and/or high concern prevails. This arena of low trust/high concern is that in which risk communication skills become essential.

Typical low trust/high concern communication opportunities include hostile telephone calls and public meetings; especially those that center around environmental issues or concerns. To these, you can add your own list of situations in which trust is low and concern is high.

This is compared with more "normal" communication situations in which high trust and low concern prevail. High trust, meaning your audience trusts your performance and credibility and low concern meaning your audience has relatively little concern about negative impact of your message or actions on what they value. Examples of high trust/low concern situations include university lectures and professional conferences, seminars or workshops.

This brings us to the three key messages at the heart of effective risk communication. Those key messages are first, *perception* on the part of the public is *reality* for that public; second, the *goal* of risk communication is to establish and foster *trust and credibility*; and third, the ability to *communicate* effectively is a *skill*, something that can be learned and developed as opposed to a talent or gift. Let's look more in depth at each of these key points. First, let's look at the idea that perception equals reality.

The fundamental principle in this statement is that what is perceived by the public as real, is real to that public; especially in it's consequences. This is especially significant in the environmental arena where one's perceptions of health effects may or may not be founded on scientific evidence or measured or measurable exposure. However, the fact that the public perceives an exposure makes it important for us to communicate with that public from their perspective.

Effective communication on the part of the scientist or government spokesperson depends in large part on the knowledge and understanding of perceptions. It also relies on the spokesperson's willingness to accept those public perceptions as real for that public. This can form the basis for message development.

Interestingly, perceptions form fairly quickly. This means the public will determine early in your presentation whether you are credible, whether your information is truthful in their eyes, and whether your message supports their particular view or views. Because

of this, we need to work toward developing our next key point; building trust and credibility.

Since our goal is to develop trust and credibility, this is an area we should address more in depth. Because, in environmental communication situations, trust is generally low and concern is generally high, only by developing trust and credibility on the part of your public can you ensure your message is heard. Of particular importance to acknowledge is that initial trust and credibility are quickly assessed. Remembering that perceptions on the part of the public are that public's reality, that early assessment of credibility is critical to your success in communicating your message.

Once you've established initial trust and credibility, you can then proceed to foster more long-term credibility. This is done, though, over the long term by continuous involvement with your public. Four elements make up the foundation of this trust and credibility pie. They are demonstrating honesty and openness with members of your public; demonstrating empathy and caring about their concerns; showing dedication and commitment to those things that concern them; and by showing a level of competence and expertise in addressing and managing those things that impact their concerns. These four elements need to be demonstrated not only on the personal level, but throughout the organization, to foster organizational credibility.

Of the four, the dominant factor to establishing and maintaining trust and credibility in a low trust and high concern atmosphere is empathy and caring. A full half of credibility,

both personal and organizational, comes from the public's perception that you are empathetic and caring, especially about their environmental concerns. The remaining half of the credibility pie is divided roughly equally between honesty and openness, dedication and commitment, and competence and expertise.

Interestingly, of these four elements, it is empathy and caring that is assessed very early in the communication process. For this reason, one of the primary teaching points in environmental risk communication education is to establish your empathy with your public before you do anything else. While different professionals offer different approaches to establishing credibility through sincere demonstration of empathy, there are several points that appear and reappear in the literature. These points include:

- ⇒ Know your public. Be involved in the community and with the community. Survey and profile the community to identify their concerns and be prepared to address those in an atmosphere that demonstrates your individual and organizational caring.
- ⇒ Address the key concerns of the public. By surveying the community and being involved in the community, you're in a position to not only know their concerns, but to address their concerns in an honest and open approach.
- ⇒ Listen to the public, figuratively and literally, and maintain eye contact. Listening, not just hearing, demonstrates you care about the public's concerns. Maintaining

eye contact further enhances, through non-verbal means, a sense of caring and empathy.

⇒ Share the frustrations of the public. By being involved, you prevent the view that you're just the government coming in on a job and moving on without making a long term commitment to the community. Many communities are built on multiple generations of residents. Trust is earned.

Demonstration of competence and expertise as it relates to the public's concerns is important in establishing and supporting your credibility with the public. This is especially true in a communication environment in which trust is low and concerns run high.

To demonstrate your competence, be prepared to state your experience as it relates to the identified concerns of the public. Practical experience makes your involvement believable as a spokesperson. Provide your credentials, whether academic or vocational, that indicate your involvement in and expertise on the topics of concern. Advanced degrees, published works, professional association memberships, when they support your topic, lend credibility to your message.

Be prepared, especially for the unexpected. Depending on what literature you read, you can anticipate between 75 to 95 percent of the questions and concerns of your public. To be this prepared requires a considerable amount of effort on your part as

well as that of your organization; but the effort all goes toward working with your public and fostering trust in you and your organization. From a practical perspective, in the low trust/high concern arena, avoid speaking from notes, from on a stage, and from behind a lectern or speaker/panel table. All of these detract from perceptions of your competence as a prepared spokesperson for your organization and as a caring, trustworthy member of the community. The message these actions transmit is that you are just a talking head, here to give the party line without regard for the needs and concerns of the community.

Honesty and openness is an area that especially a hostile public will be skeptical of. However, remember again that perceptions are reality. And one of our goals here is to develop and foster the perception, based on fact, that we are being open and honest about the issues and the publics' concerns.

Several fairly simple points can be used to enhance this area. First, if you don't know an answer to a question, make sure you refer the questioner to an appropriate expert for assistance. Saying you don't know the answer only leaves the questioner with the negative impression of your expertise. By referring the individual to an appropriate authority, and following up to ensure the question was answered, you demonstrate dedication to that individual and competence in getting the question answered. In addition, if you don't know the answer to a question, don't speculate on an answer. This is especially true if the topic is outside your area of expertise or authority. In short, avoid talking about an area in which you are not an authority. You can only get trapped.

Second, if you make a mistake, admit it early and either make corrections or commit to making corrections. Never hide your mistakes. Eventually you'll be found out and your credibility, as well as that of the organization you represent, will suffer. Next is one of the more difficult tasks -- avoid jargon. Jargon is that special terminology that has meaning to us, as scientists or health professionals, yet may be confusing or incomprehensible to our public. There are any number of specialized terms and phrases we use in our daily professional communication that is unique to our profession. I'll give some classic examples of jargon that we commonly use in a moment.

Dress for a public meeting in the same manner as the public expects you wear at work. That may mean a business suit or a service dress uniform. It may also mean slacks and sport coat or a nice looking casual dress. The key is to meet the public's expectations where they are. Finally, be relaxed. If you're tense or obviously nervous, the interpretation by the public is usually from a negative perspective. "Why is he nervous? Is it because he's lying to us?"

Demonstrating dedication and commitment is the fourth aspect of developing trust and credibility in a low trust/high concern environment. Recommendations to demonstrate your dedication and commitment include coming to meetings early and staying late to be sure everyone has a chance to address their concerns. Have you ever been to a meeting where the primary presenter arrived just in time to present then left as soon as

he or she was done with their part? To a potentially hostile public, the message received is that the presenter's priorities simply don't include the public and their concerns. No commitment!

Often times you can conduct tours and site visits that will familiarize members of the public with your operations and processes. This encourages an understanding of your activities and processes and shows your commitment to the public and their concerns as well as their curiosity.

Finally, attend others' meetings regularly and establish and maintain contacts within the community. Having a personal relationship with members of the public significantly enhances their perception of your dedication to their concerns.

The third leg in our triad of key messages is that Communication is a Skill. While there are any number of very talented spokespeople, their ability is not unique to them. Their ability to communicate effectively is something that can be developed.

There are four facets to developing effective risk communication skills. First, acquiring knowledge about communicating in low trust environments is critical. These skills are different from those typically used in high trust environments. There are a number of publications available to read on the subject of risk communication. There are also classes and workshops that present these specialized communication skills. The second facet to communication as a skill is preparation. As I said earlier, in preparing

to communicate a message to the public, you can anticipate as much as 75-95% of the public's questions or concerns. With that in mind, you can prepare your message to meet your needs while meeting the needs and expectations of the public.

Practice, the third facet to this skill, is a vital part of any presentation. Practice the message as well as the presentation to ensure the message you intend to transmit is, in fact, the one that gets across. And last, doing the communication process in real time, execution. This is where you put the message and the messenger together in front of the public and apply all your risk communication skills.

Knowledge, preparation, practice and execution. Developing these four aspects of risk communication are not the end of the line. While working through these skills, focus on some of the more subtle levels of communication. State your messages and conclusions in a positive framework, rather than using negative terminology, and avoid communication pitfalls. Communication pitfalls are numerous in low trust/high concern environments. Several of these communication pitfalls will be discussed in the following paragraphs.

Use a forum that encourages public dialogue rather than formal lecture-type information transfer. This allows the public the opportunity to state their concerns, vent if venting is what they need to do, and ask questions.

Traps and pitfalls abound in the risk communication arena. You can easily find yourself in one or more of these at any time in low trust/high concern environments. Your task will be to recognize these communication pitfalls and, to the extent possible, avoid them. Will it be possible to avoid all of them? Probably not. But as you approach communicating in a possibly hostile situation, you need to keep in mind that the public's perceptions come first.

Our first trap is humor. Humor is commonly used in high trust situations. In fact, humor is taught in most college speech classes as a way to begin a presentation. It tends to loosen up the audience and break the ice. In the low trust arena however, humor is often interpreted as a way to minimize the importance of the public's concerns or the importance of members of the public. Negatives and guarantees are also areas that can get you into trouble in the low trust/high concern arena. Risk comparisons, risk numbers, worst case scenarios and statements of personal belief are also communication pitfalls that can damage your credibility and your message in the low trust environment.

As you look over these traps and pitfalls, you can probably see at least one area that you are most likely to fall into. I'd like to focus on one of the most common – jargon.

Here are some terms that are commonly found in environmental communications, both written and verbal. The primary point to remember here is terms used need to pass the "twelve-year-old test". Risk communication messages should be prepared to generally

be presented to a 6th to 8th grade level. This means your terminology needs to make sense to the average 12 year old.

As we consider these terms, we should ask ourselves the question "would a 12 year old understand this term?"

- "Groundwater" – a 12 year old will probably think of water ON the ground. Is that what we intended?
- "Remediation" probably is what happened to keep my little brother in the third grade an extra year.
- Fugitive emissions" –
- "Toxicologist" –
- "Industrial hygienist" – Ask a 12 year old what fugitive emissions are, what a toxicologist is and what an industrial hygienist does and you may get a very interesting story. While that may be entertaining, is that the purpose of your communication effort?

Acronyms, the backbone of government and military communication, present their own problems. Something as simple as "CDC" has at least three interpretations within the Air Force; Child Development Center, Career Development Course, and Centers for Disease Control. What was your intent? Environmental law isn't immune to acronyms. SARA, CERCLA, RCRA, and OSHA all have specific meanings and refer to identifiable entities. Do the acronyms pass the 12 year old test though?

While, in the scientific community, 10^{-6} may be used to talk about that 1 in a million probability, to the average 12 year old it may be a matter of simply doing the basic math and coming up with 4.

“Exceed the standard” and “Below the standard” are both frequently used in our scientific communications. Both generally are used to indicate positive messages, or at least communicate that we are really doing a terrific job. Take a critical look at the message from the low trust perspective and you may see these phrases interpreted as “breaking the law” and “sub-standard”. Sometimes the fog factor completely hides the intended message. In low trust/high concern situations, this breaks down effective communications and widens the trust gap, especially in the environmental arena.

The last message that I want to leave you with focuses on non-verbal communication. In low trust/high concern communication situations, non-verbal messages provide as much as 50-75% of your message content.

Whether it's how you sit or stand, what you do with your hands, how you maintain eye contact; all non-verbals are noticed intensely by the audience. More than that, non-verbal messages are usually interpreted negatively. At the very best, you can expect only a functional interpretation of non-verbal actions. Very seldom is a positive interpretation made of non-verbal actions.

Non-verbal messages are not limited to your actions. Speaking from a stage, from behind a lectern, from a panel of experts; are all interpreted negatively. Also, your dress and appearance becomes a target for a hostile public. In short, if it can be critiqued negatively in a low trust/high concern environment, it will. No matter what your verbal message is, your non-verbal communication in low trust/high concern environments overrides that verbal message.

In summary, I'd like to return to my three main points. Those are...

First, perception equals reality in low trust/high concern situations. What the public perceives as real is real in its consequences. We need to format our effective communication messages to recognize and acknowledge those perceptions.

Second, our primary goal is to establish trust and credibility in the eyes of the public. Only through trust and credibility will our message be heard. At least half of our credibility is made up of empathy and caring, while competence and expertise, dedication and commitment, and honesty and openness round out the remainder of the trust pie.

Third, communication is a skill. It is founded on four pillars. They are first, knowledge -- learn as much as possible about this specialized area of communication. Second, preparation to communicate is essential. Anticipate questions and concerns. Third,

practice your messages and your delivery. And finally, just do it. Get out and take advantage of opportunities to communicate real time to sharpen your communication skills.

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**AN EVALUATION OF RISK COMMUNICATION POLICIES AND
PRACTICES PERFORMED BY FEDERAL AGENCIES**

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Abstract

The growth in the public's concern over a variety of environmental health risks has placed new requirements and demands on Public Health Service (PHS) agencies for information that describes and explains the nature of risk in clear and comprehensible terms. Experience has shown, however, that merely disseminating information without reliance on communication principles can lead to ineffective health messages and public health actions. This report presents the findings of a study conducted by the Subcommittee on Risk Communication and Education of the Environmental Health Policy Committee (EHPC) on how PHS agencies are communicating information about health risk, how effective these communications have been, and what specific principles, strategies, and practices best promote more effective health risk communication outcomes.

The purpose of the Subcommittee's study was to develop specific recommendations aimed at assisting PHS decision makers and health risk communication practitioners in improving their effectiveness to provide to-and receive from-the general public needed environmental health information about environmental exposures and disease. These recommendations are based on principles drawn from a series of case studies from PHS agencies on how best to improve risk communication activities.

Background and Purpose

Federal public health agencies have been on the front line of health risk communications for many years. Because a major proportion of morbidity and mortality is preventable through changes in health behavior, communication about health risks and benefits is critical. Health risk communication campaigns have been used to address many issues, including smoking cessation, the importance of lowering blood pressure, breast examination and mammography, and overall health and fitness (Centers for Disease Control and Prevention [CDC], 1991).

PHS agencies have invested considerable resources over the years in health risk campaigns intended to influence health behavior positively in America. In some instances these efforts have been touted as being effective in initiating or changing important behaviors related to health (Backer and Rogers, 1993). Yet, there is a growing recognition among PHS agencies of the need for communication methods that move beyond the "bean-counting" of information dissemination activities to include a variety of theoretical models (McGuire, 1986, Prochaska & DiClemente, 1992, and Bandura, 1986) for assessing the influence of health risk communications on health behavior change. Merely disseminating information without reliance on cognitive, behavioral, and attitudinal principles may lead to ineffective health messages, misdirected public health actions, and misleading outcomes.

In the absence of a planned strategy, the scope and quality of PHS health risk communications programs and outcomes varies tremendously. In some cases the PHS communications process has been reduced to measuring public health success in terms of the number of public service announcements (PSA) broadcast, products produced, or mailed out, rather than the most important reason for communicating, which is to facilitate to health behavior change (Arkin, 1992 and Johnson, 1989).

Communications researchers, such as Lawrence Wallack, have long challenged the notion of "packaging information in the right way" as the "perfect delivery mechanism" for informing the public about health risks (Wallack, 1981). Wallack argues that PHS agencies need instead is to adopt a sophisticated approach that integrates behavioral and communications considerations into the total prevention program planning and development process. One model put forth by Flora and Maibach uses social marketing concepts as a framework for incorporating marketing principles and social-psychological theories to better accomplish behavior change goals (Flora, Maibach, and Maccoby, 1989).

With attention focused on a growing number of health problems in recent years, federal public health agencies have found the task of communicating health risks to be increasingly difficult. Considerable research and anecdotal experience point to a lack of understanding between the sender of a health risk message (federal

agency) and the receiver (the public) (Agency for Toxic Substances and Disease Registry [ATSDR], 1991). Typically, a federal public health agency, when assisting communities/public in evaluating a potential health hazard situation, responds by examining the public health implications of the science and then works within legal and economic constraints to provide to the effected population its best assessment of the situation. That analysis, often presented in technical and uncertain terms, is sometimes poorly received by a public that (1) demands certainty, (2) is resistant to change, and (3) is particularly sensitive to risks perceived as imposed, and for which they have no control (e.g., exposures to toxic substances).

Unfortunately, when government agencies do not understand and deal effectively with public perceptions of health risks, public alarm about the risks and hostility toward the agencies increase. Agency credibility suffers and the public becomes skeptical or indifferent to the information about health risks provided by agency experts. Poor health risk communication may also lead to ineffective public health interventions. Because agency assessments of health risk and public concerns do not correlate, some public health concerns go relatively unaddressed while others command a disproportionate amount of agency resources.

Although health risk communication has been the subject of a number of earlier federal public attempts to inform the public

about health risks, little has been done to address the specific needs of public health agencies so that effective health risk communication can be more easily integrated into agency practices. As with other planning efforts, planning for communications must be sound, strategies effective, practices monitored, and results evaluated to achieve the desired objectives. For health risk communication, planning requires expertise in various fields such as program planning, evaluation, communications theory, social marketing, and public health.

The major purpose of this study was to identify health risk communication policies and practices that are used by Public Health Service (PHS) agencies and to evaluate their effectiveness in health promotion and disease prevention programs. The investigation attempted to highlight the basic premises and approaches that guide the health risk communication planning process. The study's secondary purpose was to (1) provide information to public health professionals in PHS agencies to better understand the basic principles that will assist them in fulfilling their responsibilities to provide to, and receive from, the general public needed health information about health risks and disease and (2) identify elements common to effective health risk communication practice.

Methodology and Organization

Preliminary Assessment by the Subcommittee

The Subcommittee's preliminary assessment revealed that, with the possible exception of the National Cancer Institute of the National Institutes of Health (NIH), most PHS agencies do not systematically apply agency-specific principles and standards in practicing effective health risk communication. In 1989, NCI made a significant contribution to the study and practice of health communications by developing a six-stage approach, commonly referred to as the "health communication wheel (NIH, 1989)." The major steps in the NCI wheel are designed to integrate assessments of target audience needs and perceptions at critical points in program development and implementation. A slightly modified version of the NCI wheel has been incorporated into the Subcommittee's recommendation for recognizing proven methods in health risk communications. The extent to which this and other similar communications models have been consistently applied in the design and delivery of health communications interventions is relatively unknown.

Further assessment revealed that most PHS agencies either were familiar with or had applied in varying degrees the model standards of risk communication developed by EPA. The Seven Cardinal Rules of Risk Communication, as identified by EPA, are

1. *Accept and involve the public as a legitimate partner.*
2. *Plan carefully and evaluate efforts.*
3. *Listen to the public's specific concerns.*
4. *Be honest, frank, and open.*

5. *Coordinate and collaborate with other credible sources.*
6. *Meet the needs of the media.*
7. *Speak clearly and with compassion.* (Covello, 1988)

The Cardinal Rules designed as a set of communications guidelines for federal agencies. The Rules provided guidance in defining communications objectives, organizing and managing decisions, and measuring performance, all within the health risk communications planning, implementation, and evaluation context. The EPA's Cardinal Rules provide a framework for examining the health risk communications process as part of a larger communications environment (context), and more specifically, the effectiveness of health risk communications undertaken by specific PHS agencies.

Some PHS agencies indicated that although they agreed with the basic assumptions and principles contained in the Seven Cardinal Rules of Risk Communication, they had difficulty applying them to daily health communication activities and decisions. Although many of the EPA rules seem obvious, they are continually and consistently violated in communicating with the public about health and environmental risks.

The author recognizes that there is no single prescription for achieving effective health risk communication. The Cardinal Rules represent one method for assuring that specific communications considerations are effectively integrated into the planning, delivery, and evaluation of health risk communication strategies. Further, the Cardinal Rules are not intended to suggest that a standard of health risk communication effectiveness is measured solely on the number of rules and critical elements that are performed or not performed. Rather, the manner in which the guidance contained in the Cardinal Rules should be applied will necessarily vary from case to case.

The Case Study Method.

It was clear from the preliminary assessment that further investigation was needed to identify some of the specific factors that contributed to a health risk communication message or activity's effectiveness. The Subcommittee proposed that a formal study be conducted to determine more precisely how PHS agencies were communicating information about risk, how effective these communications were, and what specific principles, strategies, and practices best promote more effective health risk communication outcomes.

Member agencies of the Subcommittee (CDC, EPA, ATSDR, NIEHS, NIH, FDA, HRSA, and NIOSH) provided examples of health risk communication activities or decisions they perceived to be effective and some examples of cases they thought had not been as effective as desired. Case selection of PHS agencies was based on topical relevance (e.g., PHS cases provided for health communications processes being studied) and feasibility and access (e.g., PHS agencies were willing to serve as the study's subjects).

Of the 10 case studies received, 7 were submitted as examples of effective health risk communication and 3 as examples of less effective health risk communication. Health risk communication campaigns of specific PHS agencies represent the study's unit of analysis. The 10 case studies are each organized into 4 sections.

- (1) *Case description* provides background information on the events or actions that preceded and occurred during the health risk communication process.

- (2) *Characterization of risk* identifies the specific health risk issue or problem, the scope of the problem, and levels of public concern.
- (3) *Health risk communication procedures* discusses the specific methods and strategies for communicating information about health risks.
- (4) *Outcomes and benefits* examines the results and the overall effectiveness of risk communication efforts.

Case Study Profiles.

The first case study discussed in this report, from the National Cancer Institute (NCI), NIH, reveals how a multimedia approach, along with improved diagnostic procedures for malignant melanoma, directly affected disease incidence rates and resulted in significant cost savings.

As NCI, the National Library of Medicine (NLM), NIH, relied on mass media, in this case, a national online information network, to inform health professionals about recent clinical trial findings and studies of medications and new procedures. Immediate access and the relative speed with which information could be disseminated were perceived as more advantageous than other forms of communication such as press conferences and direct mailings.

The third case history was contributed by the National Center for Environmental Health (NCEH) of CDC. Faced with the difficult task of disseminating the findings of a study to estimate radiation doses in the community to the public, CDC made use of multiple media to explain the project and build public trust.

The fourth case study, which comes from the National Institute of Environmental Health Sciences (NIEHS), NIH, shows how the effective use of timing, message clarity, and organizational

commitment can help balance the perceptions and interests of competing audiences (in this example, public advocacy groups and commercial enterprise) in the fluoridation of public water supplies.

The fifth case study from the National Institute of Mental Health (NIMH), NIH, describes the role of an intervention program in reducing risky sexual behaviors among runaway youths in New York City who are at high risk for contracting HIV. Intervention activities specific to the communication of HIV health risks - counseling sessions, training in coping skills, and video and art workshops - showed significant increases in HIV knowledge and positive attitudes toward preventing HIV infection.

The sixth case study illustrates the role of information and education in increasing the knowledge and understanding of health professionals about the health risks of hazardous substances in the environment. *Case Studies in Environmental Medicine*, a series developed by the Agency for Toxic Substances and Disease Registry (ATSDR), are designed to (1) enhance the knowledge of health professionals about the recognition, treatment, and prevention of illness or injury of persons exposed to hazardous substances and (2) improve the ability of health professionals to communicate health information concerning hazardous substances to their patients and the concerned public. Feedback from health care professionals indicates that communication between physician and patient about the health effects of exposure to hazardous substances has improved.

In case study seven, a manufacturer of heart valves informed the Food and Drug Administration of its intent to organize an extensive media outreach and letter notification program to identify, locate, and notify patients whose implanted heart valves were defective. Statistical data had shown a higher-than-normal

incidence of valve fractures, generally resulting in death. Once contacted, patients were advised to discuss with their physicians the risks and emergency procedures to follow in the event of valve fracture and to join an implant registry for any future notifications. FDA reviewed several versions of the patient notification letter to clarify the risk communication message. Activities such as hospital record searches, media outreach, and enrollment incentives resulted in 16,000 of a possible 23,000 patients being notified.

The eighth case study shows how competing interests can affect a health communication campaign. The opposition of a manufacturer of analgesic drugs (acetaminophen) to an NIEHS study that linked acetaminophen use to kidney disease points to the need for PHS agencies to be aware of how private commercial interests may influence public concern and sentiment.

In the ninth case study, ATSDR's attempts to improve public trust and credibility in a community concerned about a hazardous waste site are described. The case involves a rural site that was included on EPA's National Priorities List (NPL) in 1986 because of public health concerns due to on-site chemical contamination. Through community organization techniques, interpersonal contact, and a variety of media strategies, ATSDR has attempted to improve its working relationship with local residents in the community. The success of the community intervention remains indeterminate.

In the tenth case study, a manufacturer of a temporomandibular jaw (TMJ) implant was ordered by FDA to conduct a patient notification program. Patient notification was necessary after clinical studies showed a higher-than-normal incidence of device failure. The manufacturer subsequently declared bankruptcy, forcing FDA to assume responsibility for conducting the notification effort. Media outreach and letter notification activities were

aimed at identifying, locating, and notifying patients. Those contacted were advised to take appropriate protective actions: having a professional evaluation by a physician; having implants removed, if warranted; and joining an implant registry established to facilitate future notifications.

A more detailed description of the cases pertaining to fluoridation and the hazardous waste site are highlighted in the editorial box.

Case Description and Overview.

The communications input-outcome process for each of the PHS case studies is shown in Table 1. The communications attributes and practices of PHS agencies are organized in the matrix according to the five major components of the communication-feedback process (source, message, channel, receiver, and outcome) (Lusch and Lusch, 1987). The process transfers information from the source (agency) to the receiver (audience). Every communication event consists of a message, a channel for sending the message, a receiver, and an outcome. The message is the information that the audience is to receive. The channel for sending the message may be mass media, face-to-face communication, or some other channel form. The receiver is the target audience.

Once implementation has occurred, the final step is to evaluate the efficiency and effectiveness of the communication strategy. Process and outcome evaluation should be conducted. Whereas the purpose of process evaluation is to measure how well communication messages, materials, and services were implemented and received by intended audiences, the goal of outcome evaluation is to measure the effects (e.g., changes in awareness, knowledge, attitudes, or behavior) of the communication activity on the target

audience. The communication outcomes are evaluated for the feedback needed to improve the health risk communication process.

Table 1. Health Risk Communication Process Matrix

Source (Agency)	Message (Content)	Channel (Process)	Receiver (Audience)	Outcome	Evaluation
NIH/NCI	Improving detection of melanoma	Audiovisuals Videotapes Pamphlets Slides	General public Health professionals Patients Clinicians	Reduced incidence rates Cost savings in surgery and hospitalization	Impact
NIH/NLM	Rapid dissemination and access to vital health information	MEDLARS Network	Health professionals Librarians	Praise, concern, and disappointment from health care community	None
CDC/NCEH	Exposure to radiation doses	Printed materials 800 number Media outreach Public meetings	Community around Hanford Nuclear Facility	Build community trust and support	Process Outcome
NIH/NIEHS	Fluoride's overall risks and benefits	Professional journals Public communication	Communities Public advocacy groups	Allay public concerns Prevent cessation of fluoridation programs	Informal

NIH/NIMH	Reducing risky sexual behaviors	Videotapes skills training Private counseling	Teenage runaways	Increase in condom use Reduce high-risk behavior patterns	Process Impact

Table 1. (Cont'd.) Health Risk Communication Process Matrix

Source (Agency)	Message (Content)	Channel (Process)	Receiver (Audience)	Outcome	Evaluation
ATSDR	Environmental education and information for health professionals	Case Studies in Environmental Medicine series	Health care professionals	Enhance communication between physician and patient Improve diagnostic and treatment skills	Process
FDA	Identify and alert heart valve patients	Press releases Press conferences Notification letters Journal advertisements	Patients Consumers Health professionals Manufacturers	Identified and notified 16,000 of 23,000 patients	Process
NIH/NIEH S	Chronic drug users are at risk for developing kidney disease	Press releases Background material Newspaper articles	Consumers Physicians Manufacturers	Create awareness about risk of analgesic use	Unspecified

ATSDR	Explain science Build trust and credibility	Public sessions Community organizations Interpersonal contact Media outreach	Community Environmental activists Other federal agencies	Increase public trust and support	Process
FDA	Notify and alert TMJ patients	Media outreach Press releases Journal advertisements Notification letters	Patients Health professionals	Patients motivated to action (i.e., joined registry, contacted physician)	Under way

EXAMPLE OF HEALTH RISK COMMUNICATION PERCEIVED *EFFECTIVE* - National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health (NIH): Committee to Coordinate Environmental Health and Related Programs (CCEHRP) Report on Fluoride Benefits and Risks

Case Description

Responding to the findings of a National Toxicology Program study showing "equivocal" evidence of sodium fluoride's carcinogenicity in male rats, the Assistant Secretary for Health directed CCEHRP to prepare a report to evaluate fluoride's overall health benefits and risks.

Characterization of Risk

Although water fluoridation is known to be effective in reducing the risk for dental caries, questions have been raised about the possibility of adverse effects. Problems potentially associated with exposure to fluoride include osteosarcoma, bone fractures, dental fluorosis.

Health Risk Communication Procedures

CCEHRP released a report in February 1991, stating that optimal water fluoridation should be supported because it is safe and very effective in preventing dental caries. Some public advocacy groups expressed concern about some of the report's findings, pointing to possible harmful health side

effects such as osteosarcoma, a rare bone tumor. Although no conclusive evidence was found to support an association between osteosarcoma and water fluoridation, there is evidence linking a rise in the prevalence of dental fluorosis to high levels of fluoride exposure. Overall, the report concluded, "The Public Health Service should continue to support optimal fluoridation of drinking water."

In addition to the press releases announcing the CCEHRP report's findings, the Assistant Secretary for Health authored articles in professional medical journals further emphasizing the importance of the study. A special issue of CDC's *Morbidity and Mortality Weekly Report* was devoted to the CCEHRP report. Several key lessons were learned during the time period of the public communications campaign.

- Timing of a report's release to achieve maximum effectiveness is a critical consideration.
- Message clarity, accuracy, and balance are important when crafting a message. The fluoride report and attendant press releases were clear, as reflected by the accuracy with which they were reported in the press.
- Commitment at the top of an organization to a careful and objective response and to a fair and open process, and

the development of a clear and balanced message will greatly assist in risk communication efforts.

- Scientifically and technically complex reports may result in some messages being readily understood and acted on, while others may be lost. Because of this possibility, the need for additional educational efforts should be periodically reevaluated.
- Risk communication efforts by public agencies are facilitated when these public agencies are perceived as being objective and acting in the public interest.

Outcomes and Benefits

In the days and months after the release of the report, CDC's Division of Oral Health observed no major disruption of water fluoridation programs. Informal assessment shows that the CCEHRP report was successful in preventing communities from halting water fluoridation programs. After the report's release, there was a slow and steady increase in the number of communities providing fluoride in drinking water. On the other hand, the CCEHRP report did not appear to result in a surge of communities eager to add fluoride to the drinking water. Overall, the risk communication efforts were effective in allaying public concern about water fluoridation and in preventing a cessation in community water fluoridation programs.

EXAMPLE OF HEALTH RISK COMMUNICATION PERCEIVED LESS EFFECTIVE -
Agency for Toxic Substances and Disease Registry (ATSDR) - National
Priorities List (NPL) Hazardous Waste Site Status Report*

Case Description

The site of an abandoned chemical manufacturing plant in a southern state was contaminated with several volatile organic compounds (VOCs). The manufacturing plant produced a wood preservative by mixing diesel oil and pentachlorophenol.

As a result of a 1984 investigation of the site by EPA, the site was included on EPA's National Priorities List of Superfund sites in 1986. In 1988, citizens near the site requested a study to assess the health of local residents. The objective of the study was to determine whether the prevalence of specific diseases or symptoms for the approximately 5,000 people living within a 1-mile radius of the site differed from that for a comparison population not situated near the site.

A preliminary public health assessment was prepared by ATSDR in 1988 and was completed in 1989. The community was skeptical of the study's findings because they were based primarily on environmental information provided to EPA by the manufacturer of the wood preservative. A second study

* (ATSDR is conducting an ongoing review and investigation of the information available about contamination the site)

attempted by ATSDR in 1989 was met with similar community distrust, even though the disease- and symptom-prevalence study was conducted in cooperation with the state's department of health.

Characterization of Risk

In the past, contamination has been detected on-site at levels which, if exposure to those contaminants occurred, could pose a potential concern to public health. Soils, surface water, sediments, and groundwater are contaminated by VOCs, heavy metals, polynuclear aromatic hydrocarbons (PAHs), and various other organic compounds. Low levels of chlorinated dioxins have also been detected on-site.

On the basis of the information reviewed to date, ATSDR concluded that a potential risk to human health could result from possible exposure to hazardous substances at increased levels.

Health Risk Communication Procedures

ATSDR is faced with challenges in health risk communication in the subject community on two major fronts: (1) explaining to local residents the difficult nature of the health studies of their community; and (2) changing strongly held assumptions and attitudes in the community about alleged government malfeasance, the role of big business and

environmental racism, the right to health and health care, and a host of related concerns.

To meet these challenges, ATSDR has initiated a broad range of communication strategies and interventions. The purpose of the communication activities is to work closely with local residents in assessing community needs and concerns and then to respond to those needs by providing timely and accurate information. Many of the ongoing communications interventions have already been proven effective in working with the community to negotiate serious problems and issues and defuse some potentially volatile situations. Community involvement has increased through the following types of community organization, interpersonal contact, and media strategies:

- Community input into the design of health studies has been actively solicited by ATSDR through the Community Assistance Panel (CAP) process, which allows the community to participate directly in ATSDR's evaluation and ensures that community concerns are addressed in any ATSDR report.
- As part of the public health assessment review and consultation process, ATSDR is holding two informal public availability sessions to learn more about community concerns related to the site.

- Before each public availability session, ATSDR staff has face-to-face discussions with community members to determine their immediate information needs and health concerns. ATSDR has worked with the local media to correct scientific inaccuracies being published by some area media.
- ATSDR involved other government agencies in its activities to avoid sending conflicting messages to the community.

Outcomes and Benefits

ATSDR has learned important lessons about the attitudes and perceptions of community members toward hazardous substances as a threat to human health. Some of the lessons could be described as common sense, yet they are often overlooked:

- Take the concerns of the community seriously by responding quickly and appropriately to questions and problems. Regular follow-up is also essential for effective communication.
- Work closely with initial contacts in the local system to avoid the negative image of a meddlesome outsider.
- Emphasize personal approaches to dialogue, such as meeting people either one-on-one or by telephone, which may be more successful than a public meeting.

- Provide information that is simple and understandable. Speak in terms that people can relate to and understand.
- Use creative media sources, such as videotape, to inform the community about the purpose and activities of the agency.

Results

Analysis of the 10 case studies reveals the varying perspectives of PHS agencies on the content, implementation, and expected outcomes of health communication strategies. The data were collected and analyzed using case study methods suggested by Robert Yin (1993 and 1989) to compare the respective agencies' health risk communication strategies and practices with EPA's Seven Cardinal Rules of Risk Communication as the study's conceptual framework.

Yin's case study methods were selected for several reasons. First, the focus of the proposed study is well suited to the purpose of the case study method, which is to define topics broadly and not narrowly, cover contextual conditions, and rely on multiple and not singular sources of evidence. Second, because of its power as a knowledge-development tool, the case study method is appropriate for phenomenon (e.g., determining when communications activity started or ended) not readily distinguishable from its context or when problematic definitions (e.g., health risk communication) persist. Third, the case study method has been

shown to be an effective research method in a wide range of communication-specific settings.

Frequency counts of EPA's Cardinal Rule critical elements (examples shown) were tabulated on both a cross-case and individual-case basis. Each Cardinal Rule has a number of critical elements, totaling 43 critical elements for all seven Rules.

Cardinal Rule 2: Plan carefully and evaluate your efforts

CRITICAL ELEMENTS
Establish clear and explicit risk communication objectives
Classify and segment various groups among audiences
Aim communications at specific subgroups in audiences
Provide sufficient information to discuss risks
Recruit competent spokespersons
Train staff in communication skills
Pretest messages
Evaluate efforts

Descriptive statistics, such as means and percentage distributions, were calculated to examine the tabulations and their relationships further. Results of that analysis are shown in Tables 2 through 4.

Table 2 shows the percentage of critical elements that were included in each of the case study activities. Some of the lower percentage values (i.e., 14% and 23%) attributed to a couple of the case studies were due in part to a lack of sufficient information. Without the necessary information, the study is limited in making precise judgments or statements about health risk communication effectiveness. With those few exceptions, enough information and data were available on the critical elements to show that most PHS agencies recognized the importance of, and need for, a systematic approach to health risk communications.

Table 2. Percentage Distribution of EPA Cardinal Rule Elements
Used Within Each PHS Agency's Health Risk Communication
Activities

Case Study	Total Number of Critical Elements	Number of Critical Elements Included in Activity	Percentage
1	43	10	23%
2	43	6	14%
3	43	27	63%
4	43	33	77%
5	43	23	53%
6	43	30	70%
7	43	34	79%
8	43	14	33%
9	43	27	63%
10	43	21	49%

Table 3. Mean and Percentage Distribution of EPA Cardinal Rule
Critical Elements Used Across PHS Agencies' Health Risk
Communication Activities

EPA Cardinal Rules	Critical Elements	Mean	Percentage
1	2	1.8	90%
2	8	5.4	68%
3	4	2.3	58%
4	10	7.1	71%
5	5	3.1	62%
6	5	2.8	56%
7	9	5.0	56%

Table 4. Effectiveness of PHS Health Risk Communication
Practices in Following EPA's Seven Cardinal Rules of
Risk Communication

Case Study	Highly Effective Cardinal Rule	Moderately Effective Cardinal Rule	Least Effective Cardinal Rule
1		2, 6	
2	5	6	2
3	1	3, 5, 6	2, 4, 7
4	1, 3, 4, 5, 6	7	2
5	1	2, 3, 5, 7	4
6	1, 2, 4, 5	3, 7	
7	1, 3, 5, 6	2, 4, 7	
8	5	4, 6	2, 7
9	1, 3, 4	5, 6, 7	2
10	7	2, 3, 5, 6	4

Table 4 takes the analysis results in Table 3 one step further, to look more specifically at the Cardinal Rules that figured prominently in health communication activities of PHS agencies. On the basis of percentage distributions, the Subcommittee was able to group the Cardinal Rules for each case into the three broad categories of "highly effective," "moderately effective," and "least effective." A frequency count of the Cardinal Rule critical elements provides a revealing set of general characteristics associated with health risk communication effectiveness. An analysis of the data in Table 4 reveals that in the "highly effective" category, Cardinal Rules 1 (*Accept and involve the public as a legitimate partner*) and 5 (*Coordinate and collaborate with other credible sources*) seem to be common to most PHS agencies. In the "moderately effective" category, Rule 6 (*Meet the needs of the media*) is found most frequently. In the final category of "least effective", Rule 2 (*Plan carefully and evaluate efforts*) occurs most often.

Content, Process and Outcome Evaluation

A qualitative analysis of the case studies reveals varying beliefs among PHS agencies about what the content, process, and outcome of a health risk communication should be. To simplify the analysis, the EPA Cardinal Rule critical elements were broadly grouped into the recognized communication categories shown in Table 1: communication content, communication process, and evaluation of

communication outcomes and impacts. Using this schema, PHS agencies were able to identify some perceived strengths and weaknesses of their health risk communication practices.

Communication content, the first component, refers to how relevant and salient the information or message is to the receiver's information needs and concerns. The amount of emphasis given to content varied substantially among the agencies. FDA, for example, expended a great amount of effort in content analysis and the pretesting of messages. Some agencies, on the other hand, chose to create messages with little audience input. Gaps in the content component of health communication campaigns were evenly distributed across the agencies.

Communication process is the second and most labor-intensive of the components. During this stage, the rationale for choosing specific health risk communication strategies and techniques is proven either effective or ineffective. Most of the agencies that submitted case studies are involved in health risk communication activities to some extent. Yet, few of the agencies could clearly explain the rationale and content of their health risk communication goals and strategies. Several factors contributed to this condition. First is an inadequate recognition that audiences differ greatly in value systems and levels of involvement in the health risk communication process. FDA provided one exception to this trend by conducting focus tests with their audiences to

identify relevant needs and expectations. Second, systematic methods for defining health risk communication needs and responsibilities were unspecified. Third, discussion about the role of health risk communications as a function of agency mission, goals, and objectives is too limited. Finally, barriers to the effectiveness of health risk communications may be internal, such as constraints of staff, resources, and budget, or external, such as the activities of interest groups or the limits set by policy or mandate.

Having to explain health risk and uncertainty were common experiences shared by most of the agencies. Yet the specific procedures that were used in the various risk events differed according to the type of health risk issue, the scope of the problem, and levels of public concern. CDC, in its Hanford Environmental Dose Reconstruction Project, provides an excellent example of how, when properly implemented, the process can achieve the desired outcomes. CDC very effectively organized its health risk communication campaign around the simple principle of using multiple media strategies to build community trust and support.

The last component, *outcome and impact evaluation*, is an ongoing and systematic procedure for assessing the efficacy of health risk communication strategies in achieving intended outcomes. Evaluation was the least understood of the communications components. Collecting process and anecdotal information was the

preferred method of evaluation. However, to properly judge the effect of a health risk communication activity, measurable objectives must be designed in the planning stage and tracked until the completion of the activity.

One of the evaluation successes involved NIMH and its study of reducing sexual risk behaviors among runaways. Basic evaluation rules were put in place before, during, and after the intervention program. Both qualitative and quantitative evaluation measures were used. With these measures, NIMH was able to assess audience perceptions, track the achievement of communication objectives, and improve the quality of services provided to runaways.

Recommendations for Improving the Effectiveness of Health Risk Communication

PHS agencies were able to identify a number of areas for improvement in their attempts to design and implement effective health risk communication campaigns. The following recommendations emphasize the need for both short- and long-term actions to improve health risk communication planning and practice.

Area One for Improvement: Increased understanding of the health risk communication process and its importance in achieving agency mission, goals, and communication objectives.

Specific Recommendation: PHS agencies should consider developing a focus specific to health risk communication. One

approach would be to form an office or designated group within each agency to help identify and clarify the role of health risk communication beyond its traditional function of information dissemination. A major task of the office or group would be to create an awareness of health risk communication as an integral agency component, interrelated to other functional areas and well established within the larger planning budget process. Developing a systematic approach to health risk communication process planning and management can enhance an agency's awareness and recognition level.

Area Two for Improvement: Lack of a systematic approach to planning leads to poor conceptualization and execution in the preparation, production, and dissemination of health risk communication messages, materials, and campaigns.

Specific Recommendation: Each PHS agency should develop a set of generally accepted practices or guidelines for effective health risk communication, perhaps using the EPA Cardinal Rules of Risk Communication or organizing a consensus conference of communication experts and practitioners to set standards for health risk communication. Agencies should consider using these case studies to provide a starting point for identifying guidelines for their own health risk communication initiatives and activities. Some of the basic principles contained in the case studies for improving health risk communication include the following:

- Ongoing communication, information dissemination, and follow-up build public trust and support for risk communication activities.
- Involving the audience early in communication, planning, and problem-solving processes enhances the efficacy and acceptability of the intended message.

- Active listening and recognition of verbal or nonverbal cues build credibility and empower the audience to create its own agenda.
- Releasing information that is timely, accurate, and understandable helps allay some of the public's concerns and fears.
- Communication directed at increasing cooperation and coordination among individuals, groups, and agencies reduces competing interests of political and social groups, which can inhibit communication.

Area Three for Improvement: Lack of a well-organized and broad-based approach for increasing awareness and visibility of health risk communication issues and trends within and between federal agencies.

Specific Recommendation: The Subcommittee on Risk Communication and Education, in coordination with other PHS components, should undertake an interagency initiative aimed at increasing awareness and visibility of health risk communication issues and trends within and between PHS agencies. Possible examples of this initiative could include health risk communication workshops and focus groups jointly organized and sponsored by the Subcommittee and the Office of the Assistant Secretary for Health (OASH). The proposed initiatives provide several key benefits:

- Providing a public forum for the discussion and debate of current health risk communication issues and trends.
- Promoting broader dissemination of research to health communication practitioners within the public health community.
- Covering developments in current health risk communication trends, priorities, and practices.

- Improving coordination in linking health risk communication expertise, information, and resources among federal agencies.

Area Four for Improvement: Analysis of the case studies showed that only 3 of the 10 agencies conducted any type of formal outcome and impact evaluation. The remainder of the evaluations ranged from simple implementation measures to no evaluation at all. Not having this type of data limited the agencies' in judging the quality and worth of their communication activities and products. Also limited were judgments made in relation to statements of intended outcomes.

Specific Recommendation: Each PHS agency should develop a set of generally accepted practices or guidelines for effective evaluation of communication activities and products. Agencies should become familiar with evaluation standards and practices, perhaps by hiring evaluation specialists or by developing similar expertise among current staff members. Clear objectives should be developed for each health risk communication effort and tracked to the activity's completion.

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**ABSTRACT OF PRESENTATION:
METHYL PARATHION CONTAMINATION
OF PRIVATE RESIDENCES: RISK
ASSESSMENT, RISK MANAGEMENT,
AND RISK COMMUNICATION
APPROACHES**

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The interiors of 500 residences were illegally sprayed with methyl parathion, a highly toxic agricultural pesticide. Over 1,000 persons had known exposures to methyl parathion. Federal, state, and local agencies began a process to evaluate the risks and establish emergency relocation and decontamination priorities. The joint effort led to a risk assessment process which permitted identification of affected properties.

Communication aspects involved personal sessions with affected homeowners, letters containing information on environmental and biological levels of the pesticide, multilingual explanations of actions which health and environmental agencies would take, and relocation guidances.

Risk assessment techniques and biological monitoring identified 230 residences needing decontamination. Based upon levels of exposure, residents were either relocated on an emergency basis (48 hours), within two weeks, or within a month. All 230 residences were fully decontaminated in 14 months. No other Superfund project has decontaminated as many residential properties and protected as many people within a similar time period.

Objective:

To demonstrate how risk communication aspects permitted effective decontamination of 230 residences, involving 860 people.

**A CASE STUDY OF PUBLIC AVAILABILITY SESSION USE AT
ABERDEEN PROVING GROUND, MARYLAND**

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Abstract

Risk communication is an approach to communicating with the public which has great potential application at Department of Defense facilities with environmental problems. A case study is presented from Aberdeen Proving Ground, MD where risk communication techniques have proven useful in informing the public about restoration efforts and addressing public concerns. This case study demonstrates the use of an alternative to traditional style public meetings, where community members interact personally with installation staff. Benefits of this approach are presented, along with details tailored to a specific situation. This approach has been employed to help establish the trust and credibility necessary to address public concerns at many sites having the potential for adverse environmental effects.

Risk communication is an approach to communicating with the public which has great potential application at Department of Defense (DOD) facilities with environmental problems. It is safe to conclude that this potential has not been fully exploited. One reason for this is its dual nature. Technical people frequently are not comfortable addressing issues which they believe have no rational basis. Conversely, communications professionals often feel that they do not have the background to adequately address technical issues. Failure to address these problems can result in poor relations with surrounding communities, needless anxiety for residents and, potentially, demands for unwarranted responses to perceived risks. The purpose of this paper is to provide an example of how risk communication ideas were put into action to minimize these difficulties at a high profile DOD facility.

Aberdeen Proving Ground (APG) is a 73,000 acre U. S. Army installation in Maryland, on the western shore of the upper Chesapeake Bay. Since 1917 it has been a center for testing ordnance. The Edgewood Area of APG has been used for the research, testing and production of chemical warfare materials (CWM), as well as training in their use. These activities resulted in the introduction of a variety of chemicals into the environment at numerous sites. The potential presence of CWM has added an extra dimension to environmental concerns.

Like many installations, APG is surrounded by growing suburban communities. When originally established, APG was in a

sparsely populated area and little attention was paid to activities which would conflict with neighbors. Prior to the 1970's and 1980's the majority of residents in the southern part of Harford County knew of APG as the largest employer in the area. Most either worked at APG, or had friends, relatives or neighbors working there. Consequently, there was a general knowledge about, and tolerance of, the activities that went on at the proving ground. Subsequent rapid expansion of Baltimore suburbs into the area has brought housing development to the installation boundary. Many of these new neighbors were unaware of the presence of APG and its past and present missions. However, this situation changed in the spring of 1995.

On Sunday, March 26, the headline on the front page of the Baltimore Sun read "Arms cleanup sounds alarms - Harford residents learn chemical shells are buried near homes". While the potential for chemical filled unexploded ordnance (UXO) had been a matter of public record since the mid 1970's, it had received little attention. The media became aware of the situation because of the remedial investigation of a tract of land known as the Nike Site. In the course of monitoring well installation at this site UXO had been uncovered. This information was disseminated during routine briefings to our Restoration Advisory Board, and a reporter from the Sun requested interviews with the APG staff on the UXO issue.

Alerted that an article was about to appear, the installation staff anticipated a high degree of public concern. It was decided

that it was preferable for our neighbors to get their first notice of this situation from the Army rather than the media. A letter to be sent from the installation commander to 20,000 surrounding households was carefully drafted describing the situation. It assured residents that their health and safety were our top priorities and that their input would be solicited before any cleanup actions were undertaken. The letter was mailed two days after the installation learned of the article, several days before the article actually appeared. This was key in retaining control of the message rather than having the public rely on the media for all of their information.

The lack of risk awareness in the community combined with the presentation in the media did result in a degree of public alarm. Following the appearance of the article, real estate settlements were canceled. Parents were anxious for the safety of their children, who attended three public schools near the area in question. The Army's anticipation of the need for some sort of rapid response was appropriate. In order to prevent the situation from deteriorating further, face to face communication with the public was begun six days after publication of the article.

APG was able to meet this schedule because a core group of technical and communications personnel had already received training in risk communication. In part this was due to the presence of the U.S. Army Center for Health Promotion and Preventative Medicine (USACHPPM), whose headquarters is at APG.

Starting in 1992, USACHPPM has sponsored training by Dr. Vincent Covello, director of the Center for Risk Communication. Their location gave many APG staff members an opportunity to attend this training. However, there was no unanimous agreement on which of the numerous methods presented in the course would be useful in this situation. One idea which had wide acceptance was the need for a more effective means of communication than the traditional "town hall" style of public meeting. This proved to be the cornerstone of our response.

The installation decided that it was vital to give local residents an opportunity to voice their concerns and have them answered in a timely manner. Due to the potentially large number of concerned citizens, it was necessary to do this in a public format. However, we wished to avoid a traditional meeting format in which individual speakers on a stage present information to a large audience. Traditional meetings do not provide an opportunity for everyone in attendance to have their individual questions answered. This is due to both time considerations and the unwillingness of some to speak in public. In addition, not everyone has the same questions, nor is everyone able to attend at the same time. Another beneficial aspect of avoiding the traditional format is that individuals are not able to introduce irrelevant topics into the agenda or monopolize the event (Sandman 1990; Covello 1994).

The public availability session was chosen as the format to

replace the traditional public meeting. In our interpretation of this format, which is described by Covello (1994), stations were set up with information displays staffed by knowledgeable people. Citizens have their questions answered directly and have the ability to interact and have answers clarified or magnified as necessary. In this circumstance the cafeterias of local schools were a suitable location. Notification of the sessions was made through the letter from the installation commander, as well as regular advertising channels.

The installation staff wanted to be able to greet people personally, have them sign a register in order to be placed on our mailing list, and direct them individually to the station addressing their primary concern. We also anticipated that the high level of interest would prompt large numbers of people to arrive at these sessions as soon as the doors opened. We did not want to further antagonize people by causing them to wait in line to enter, so we made efforts to obtain a large group of "greeters" who would gather initial information from people to determine their concerns as they arrived. Most of our greeters were technical people not accustomed to dealing directly with the public. This was inevitable due to the nature of our organization, but it had the potential advantage of giving citizens some personal contact with the technical people who would be working on the issues.

A requirement of this approach was the need to prepare the

greeters. We wanted the greeters to handle some of the more general questions so that citizens had a favorable impression when they arrived. Also, we did not want our more limited number of subject area experts to be confronted with more people than they could address in an expeditious fashion. In addition, we wanted consistency of responses. To this end, greeters attended a training session and received a list of anticipated common questions and answers. In addition, our risk communication goals were explained and a limited number of techniques were recommended to those with no prior training. Greeters recorded mailing list information on forms. Questions which they could not answer were also recorded if the citizen did not wish to speak to a subject area expert. They also had copies of a very carefully prepared fact sheet which covered presumed areas of common concern. We hoped that many people would be satisfied with this level of information, and, according to our exit poll, this seemed to be the case.

Staff members from the Installation Restoration program were conversant with the major issues. They were designated as "briefers" who would interact with more highly concerned citizens. Their task was to take families or other small groups aside and spend as much time with them as necessary to respond to their questions and concerns. Typically this was ten to fifteen minutes but sometimes considerably longer. The briefers could sit with the groups at cafeteria tables where they had maps available to help orient people to the area in question. These proved very

helpful. For parents with small children, we provided a babysitting area in the room so that everyone could feel more at ease.

The importance we attached to these opportunities to hear our neighbors' concerns was underlined by the presence of the installation commander and his deputy. This was especially understood by present and former employees and soldiers who knew that it was unusual for a general officer to spend an entire day at a public meeting and make himself available to talk to anyone in attendance.

Subject area experts were made available from several organizations. A medical doctor familiar with the effects of chemical agents was on hand. Emergency Preparedness personnel were there to explain their procedures. Representatives from tenant organizations such as the Chemical Demilitarization Program were present to clarify their roles on the installation. The legal office presented the official position on financial restitution. Unexploded ordnance technicians were provided from the U.S. Army Technical Escort Unit. It was especially important for the public to understand that these soldiers, the Army's top experts in dealing with unexploded chemical munitions, were based at APG and would be recovering any munitions found.

The participation of the media required additional risk communication protocols. We wanted to create a setting in which

the press would have access to information and opportunities to interview technical personnel. At the same time, we wished to manage the situation so that the event was not dominated by the media presence and citizens were not overly distracted. This was accomplished by providing a separate area near the entrance where cameras could be placed, and citizens could be interviewed. Camera crews were allowed limited access to the main room to obtain footage of the event in progress. The Public Affairs Office provided communications professionals for any direct interaction with the media. This arrangement caused no complaints by the media and met the goals of the Installation.

Attention to detail is important in making risk communication work. We chose casual dress as the uniform of the day. We did not want to be perceived as government "stuffed shirts" when compared to the citizens in their weekend attire. We avoided having greeters bunch together and discouraged chatting among the staff. Humor was avoided since local residents did not see anything amusing in the situation. A break room was provided so that staff would not be seen eating. Outside of the break room, staff remained standing unless they were talking to the citizens. Interestingly, an attempt to videotape aspects of the availability session for internal training use was strenuously objected to by one citizen. He believed that a dossier was being prepared on those in attendance. This underscores the need to anticipate a variety of points of view in the effort to generate trust in an institution whose credibility with the community is not uniformly

high.

The public's satisfaction with this format was formally gauged through the use of exit polling techniques. At the first meeting, in anticipation of the large attendance, APG decided not to use a written survey since it would delay attendees as they left. Instead, staff members asked attendees if they had received answers to their questions; responses were written down. The questioning prompted a few attendees to voice further concerns, and they were re-directed back into the room to the appropriate person for an answer. Anticipating a smaller attendance at the second meeting, the installation staff prepared a short questionnaire to evaluate the effectiveness of the availability session.

The reaction of local residents to this approach was favorable. The majority of attendees indicated their concerns were addressed and they were anticipating receiving additional information through newsletters and fact sheets. They were able to get some feeling for the people who were in charge of the remediation projects and see that they really were concerned for their well being, even if they were unable to resolve their questions or concerns. The interaction made it possible to clear up misunderstandings on a variety of topics, partly because family members could help one another clarify questions and understand answers. A copy of the form used for the exit poll is provided in the appendix with sample results from one session.

The installation also tracks the effectiveness of communications through informal feedback. Participation in community events allows individuals to easily express their opinions and helps APG monitor community sentiments. Editorials, letters to the editors, and newspaper articles are also monitored. In a column by a local real estate agent, he described his experience of attending the APG briefing on the UXO project to the local realtors association. In his own words, he entered the room with "an inherent distrust of the federal government." After hearing the presentation, he "came away convinced that APG is doing everything it can to make cleanup as safe as possible." He encouraged other members of the community to take advantage of the many information and education opportunities APG offers.

The availability session approach was viewed as an overall success. Judging by the poll and general reactions, the primary objective of reducing panic was achieved. We did not expect that we would be able to address everyone's issues to their satisfaction, but we felt that most people were glad to have an opportunity to have their questions answered on an individual basis and to have their concerns noted. There was a wide range of reactions and a few individuals resented the lack of an opportunity to publicly express their feelings, but this was a minority compared to the hundreds who attended. When given the opportunity to voice their concerns to the installation commander, most of this minority declined.

The public availability session was not the only means we employed to alleviate concern. We offered a number of opportunities to take bus tours of the installation. Even though part of the post is open to the public, many neighbors had never visited. We also showed areas which are not normally open to the public, and this was very helpful in dispelling a variety of images based on rumor. Many citizens left impressed by the pristine appearance of many of the restricted areas which they had assumed would be environmental disaster areas. Another useful element in our response was a mailing to the 20,000 households which received the original letter from the commander. This consisted of a list of the questions most commonly asked at the availability sessions and their answers. In this way, we felt that those who did not attend could still benefit from the experience.

The importance of obtaining names of those attending for our mailing list should be emphasized. Communication is an ongoing requirement, and the use of a mailing list is by far the most cost effective method of getting information to the community. In a qualitative survey sent to 6,000 community members and employees (selected through a stratified random sampling method) as part of preparing a community relations plan, 70% of the respondents selected newsletters as their preferred method of receiving information. Our surveys also indicate that the majority of people prefer to receive fact sheets specific to their interests above any other mode of information transfer. They said that if

they knew beforehand that they would only receive items of interest, they would be less likely to discard them with the junk mail.

This approach to communication was very resource intensive. Staff were required to work four consecutive Saturdays. The commander and his deputy were present for three full days. In addition, the sessions required training time during regular business hours and a large amount of time in coordination efforts. There were also considerable costs in producing displays. However, these need to be balanced against the resources needed to deal with the fallout from a less proactive approach. In addition, while this approach was exhausting for many, it was not as stressful as a highly charged traditional style public meeting. There was also the intangible benefit which the staff received from feeling that they were helping the citizens rather than merely spending time in deflecting criticism.

The most important aspect of risk communication is establishing trust and credibility with the target audience. We made every attempt to put the risks inherent in the situation in perspective. At the same time we did not attempt to deny the risks or the legitimacy of people's concerns. There is no replacement for being able to empathize with people and no amount of technical expertise can cover up a lack of sincerity.

It could be said that the events discussed above do not

reflect a typical risk communications scenario at a hazardous waste site in that potential acute effects and the fear that they generated were not very amenable to technical explanations or discussions of risk. However, it does illustrate that a core of trained personnel applying some of the principles of risk communication can help the community to overcome panic and deal with its concerns in a more realistic and productive way.

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**ABSTRACT OF PRESENTATION:
WHEN SIMPLE LANGUAGE FAILS:
ADDRESSING LAY THEORIES
THROUGH RISK COMMUNICATION**

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People often say that presenting complex scientific information to the public essentially involves editing scientific jargon and using short, easily understood sentences. However, a substantial body of research in science education indicates that traditional explanatory tools such as simple words, short sentences, examples, and analogies are useless for overcoming an important obstacle to the understanding of science. This obstacle is that many fundamental principles of science are counter-intuitive. For example, people have difficulty believing that they need to wear seatbelts in part because they do not understand concepts of gravity, inertia, and force. Similarly, people cling to naive and inaccurate theories of disease in part because these naive notions seem supported by daily experience.

To assist professionals in detecting, diagnosing, and overcoming lay theories frequent in risk communication contexts, this presentation (a) describes the class of scientific ideas that the public is most likely to find counter-intuitive, (b) reviews research specifying techniques for overcoming lay theories, and (c) suggests when and how these techniques might be best used in communicating about controversial or complex physical hazards.

Risk Assessment: What it is to the Government, What it is to the Community, and What it Should Be

by Marie T. Flickinger

The Houston Brio Toxic Waste Site, active during the 1950's through early 1980's, is a garbage-collection dump of 26 earthen unlined pits. These pits accumulated numerous types of chemicals from chemical-generating companies; such as Monsanto, the primary contributor. Through research associated with Houston's Brio Toxic Waste Site, serious problems were identified and associated with Brio that are not uncommon when it comes to Risk Assessments.

In 1989, the U.S. Public Health Service's Agency for Toxic Substances and Disease Registry (ATSDR) issued a Health Assessment (based on Brio's Environmental Protection Agency Risk Assessment) which stated, "based on the available information the Brio/DOP sites currently pose no significant risk to public health . . ." ⁽¹⁾. There was no recommendation for sampling of air; later proven to be the major route of exposure.

EPA's risk assessment was the basis on which the community trusted the government, and allowed itself to be potentially exposed for a number of years. Based on "no-reported" risk, the Little League, with 1,000 children, continued playing ball across the fence from Brio---after all, the government said it was safe.

Residents were repeatedly assured that it was safe to live in the adjoining 667-home Southbend subdivision, some just 18 inches from a pit containing 22,700,000 ppb's of vinyl chloride and many other chemicals. When residents became concerned and moved due to health problems or fear, many young minority couples bought bargain homes too-good to be true. All the while, EPA, federal, county and state health officials, as well as the chemical companies, assured everyone it was safe.

The school district officials relied on EPA, ATSDR, and local health officials in making decisions concerning the children attending the elementary school located just 800 feet from Brio. Not only were parents not allowed to transfer their children to another public school, children from distant communities were bussed into the school. The school remained open, based on a totally inadequate Risk Assessment. State of Texas air monitoring atop the elementary school later proved air exposure did occur from the site. Brio became the superfund site that the National EPA Ombudsman called "the poster child for what is wrong with Superfund."

What is the status of that vital community today? The ballpark is deserted, overgrown with weeds---only a couple of backstops and score booths are barely visible to those passing by, where so many young ball players spent their spring and summer months.

The subdivision, once occupied by newly-married couples starting families, and a few senior citizens who had found the perfect place in which to retire, had a life of less than 15 years. Most homes sold well below normal property values. One 2000 sq. ft. house sold for less than \$40! The homes are boarded up and in various stages of disrepair. The local swimming pool has been filled. Soon, the recreation center and homes will be bulldozed and will become fodder for a landfill.

The \$8M elementary school is boarded up and awaits the same fate. The streets are barricaded. An armed security guard discourages inquisitive visitors from entering the subdivision.

Without question, the basis for this environmental travesty was a totally-flawed risk assessment.

The government conducted Brio's risk assessment like those at many potentially-hazardous sites. Hazards and pathways of exposure are evaluated, usually from written information supplied by those responsible for the environmental problem. Potential receptors, if any, are identified. Modeling is then performed.

Modeling was done at Brio--but the information was faulty. Consequently, thousands of people, many of them children, were put at risk. Many reputable physicians have confirmed that children have died and have suffered birth defects due to exposure to Brio.

What went wrong with Brio's risk assessment?

Everything.

Who is to blame...Congress, government, industry, community?

All of the above are to blame.

Suffice to say, Congress does not find many solutions to problems; they just muddy the waters. So it was with Brio and hundreds of other waste sites, Congress mandated ATSDR to perform approximately 1,000 health assessments in about a year's time. An impossible task to do properly.

Governmental agencies such as EPA, ATSDR, and county and state health agencies are too quick to accept the easy answer. In the case of Brio, everyone believed the chemicals were confined inside the site boundary lines. EPA's Project Manager for Brio stated it was "OK" for kids to be playing ball on their fields. Less than 50 feet away, contractors were in full protective gear. Why? Because there was a fence between the properties...a chain link one at that.

Industry is to blame, because many are more concerned about the bottom line and possible litigation. Consequently, it is beneficial not to identify problems of which only they may be aware.

Community is also to blame. Doctors are reluctant to admit they may not be aware of effects of chemical exposure in the community and realtors and other business people would rather hide their head in the sand. Family providers are reluctant to accept they have put their family at risk. Therefore, a situation like Brio had to get totally out of hand before the problem would accurately be identified and corrected.

In the case of Brio, all federal, state, and county agencies professed there was no exposure due to contaminated air, soil, or water. This was believed by most for nearly 10 years--and all this time, the community was at potential risk of exposure.

Those same agencies now acknowledge exposure occurred through multiple pathways. Brio chemicals have been found under homes, in yards, in the nearby creek (which was closed to fishing), in the air, and even in water from the subdivision's drinking well.

To understand in the early days of Brio, doctors and the government did not have the answers. Two or three years earlier, our local newspaper provided our readers the citizen survey which showed a measurable rise in birth defects when EPA and the Brio PRPs conducted site work. It was attributed to chemicals in the air caused by soil disruption. We were scoffed at by

those doing the site work. Our community study showed 11 of 13 babies born with life-altering birth defects were by mothers who had been exposed in their first trimester during four months of extensive site work. In all likelihood, these children would not have been exposed if a proper risk assessment had been done for Brio. Why would anyone with common sense live next to a toxic waste site? With chemicals that were mutagenic and more, why would anyone knowingly live next to such a place that presented the possibility of increased birth defects which science had already acknowledged.

ATSDR's Dr. Barry Johnson stated that, "knowledge of community exposure to toxic chemicals is where industrial exposure was 20 years ago."

Doctors, government, and even scientists did not have the answers for such problems as additive and synergistic effects of chemicals. Brio is an alphabet soup of chemicals. Even today, we cannot rely on predictions to be accurate regarding effects of exposure to this type of hazard.

While trying to remove Houston mold from a shower door, I once used Comet and elbow grease without achieving the desired effect. I then added Clorox. I found that you can use products such as these independently and suffer no ill effects. When mixed, any two chemicals in an enclosed area can result in the need for fresh air circulation.

A report in the local Houston Chronicle indicated the combined affects of independently-safe chemicals may be causing the Gulf War Syndrome. Do chemicals, mixed together, cause toxic problems more so than each one independently? Many reputable scientists believe in some cases "yes"; yet, it is very difficult for risk assessments to reflect this concern.

Based on our community health survey, two professional studies done at the request of ATSDR and a third by ATSDR confirmed our worst fears. It was concluded, due to evident health effects, that the risk assessment environmental data was faulty.

Dr. Waldemar Johanson's thesis study ⁽³⁾ on our citizen birth defect survey concluded that the most conservative risks showed 2.4 times and 3.8 times the national statistics for congenital heart and central nervous system defects, respectively. This indicated that the abnormalities were much more likely to occur at Southbend subdivision than elsewhere in the U.S. Respiratory complaints in the subdivision increased in frequency with proximity of residence to the pit area ⁽³⁾.

Due to the results of Johanson's study, ATSDR commissioned Dr. Maria Morandi of the University of Texas School of Public Health to do an environmental data review... the review the community had requested for years.

Dr. Morandi's review ⁽⁴⁾ supported the community's accusations that the environmental data in the risk assessment was flawed. Morandi's report characterized the risk assessment data as insufficient and inappropriate for assessing risk from exposure to air, water, and soil. Dr. Morandi concluded, "Therefore, it is not surprising that the characterization data, available for Brio, are generally deficient for application in exposure assessment."

Subsequently, ATSDR reopened Brio's health assessment and conducted biological testing of the residents and a similar neighboring community. ATSDR biological testing supported increased odds for the Southbend residents to have UAAP, IgG, and MCV levels outside the reference range. ATSDR recognized air as the primary pathway for exposure ⁽²⁾.

Today's technology is capable of assessing risk much more accurately. No longer should a community be put at risk by written evaluations performed by government and/or industry. When possible, exposure testing should be done. At Brio, air monitoring proved exposure. Tests on fish showed volatiles in fish flesh; never seen before in Texas. Soil samples revealed homes were built on dense non-aqueous phase liquids (DNAPLS) with high levels of vinyl chloride, dichloroethane, and trichloroethane.

The community was being exposed. Had proper testing or a good risk assessment been performed, the facts would have been obvious. Exposure could have been addressed years earlier. The elderly, the children, the unborn and their parents would not have been exposed. Civil litigation cost is nearing a billion dollars at Brio. Legal costs could have been greatly reduced, if the number of people potentially exposed would have been greatly reduced.

By definition, now is the time, to bring risk assessments into the 21st century, by directly applying science when proven scientifically, such as this, everyone benefits.

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ABSTRACTS FOR POSTERS

POSTER SESSION ABSTRACTS
CONFERENCE ON ADVANCES IN TOXICOLOGY AND
APPLICATIONS TO RISK ASSESSMENT

(PRESENTER UNDERLINED)

DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR TRICHLOROETHYLENE AND ITS METABOLITES IN B6C3F1 MICE

R. Abbas, J.W. Fisher, R.K. Black, T.J. Janicki, and K.L. MacMahon
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OXIDATIVE STRESS AND PROGRAMMED CELL DEATH OF CULTURED J774A.1 MACROPHAGE CELLS IN ASSOCIATION WITH CADMIUM AND CHROMIUM IONS

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ASSESSING HEALTH RISK ASSOCIATED WITH INDIRECT EXPOSURE TO COMBUSTOR EMISSIONS: RE-EVALUATED

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ECOLOGICAL RISK ASSESSMENT OF A HAZARDOUS WASTE SITE IN THE MOJAVE DESERT OF CALIFORNIA

Jeffrey L. Briggs, Glen J. Barrett, and Robert L. Sandoli
Earth Tech Corporation, Alexandria, VA

COMPUTER-AIDED DESCRIPTION OF CHEMICALLY INITIATED OXIDATIVE STRESS *IN VITRO*

J.Z. Byczkowski and C.D. Flemming
ManTech Environmental Technology, Inc., Dayton, OH

DETERMINATION OF BLOOD:AIR PARTITION COEFFICIENTS FOR POORLY SOLUBLE CHEMICALS

M.C. Caracci, A. Vinegar, and G.W. Jepson
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DETAILED SOIL SURVEY IN ECOLOGICAL RISK ASSESSMENT: LOCATING BACKGROUND SOIL SAMPLES TO REDUCE UNCERTAINTY AND COST

Ronald T. Checkai
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UPTAKE AND PHYTOPHYSIOLOGICAL RESPONSE OF CROP SPECIES TO IRRIGATION WATERS CONTAINING LOW CONCENTRATIONS OF RDX AND TNT

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SELECTED APPLICATIONS OF REDUCED UNCERTAINTY FACTORS IN NONCANCER RISK ASSESSMENTS

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TRICHLOROETHYLENE METABOLISM IN HEPATIC MICROSOMAL AND S₉ PROTEIN OF THE JAPANESE MEDAKA

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IN VITRO EFFECTS OF AMMONIUM DINITRAMIDE

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EVOLUTION OF SCIENCE-BASED UNCERTAINTY FACTORS IN NONCANCER RISK ASSESSMENT

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PREPARATION OF BOVINE TESTICULAR SLICES: EVALUATION OF TRINITROBENZENE TOXICITY

John F. Wyman, Jeffrey S. Eggers, Linda Steel-Goodwin, Carlyle D. Flemming, and Daniel J. Caldwell

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SEVEN DAY DOSE RANGE FINDING STUDIES FOR TOXICITY ASSESSMENT OF LONG CHAIN PETROLEUM HYDROCARBONS

D.H. Ellis, D.E. Dodd, R.E. Wolfe, and W.H. Weisman

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A BIOLOGICALLY BASED KINETIC MODEL FOR THE ISOLATED PERFUSED RAT LIVER

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COMPARISON OF THE *IN VITRO* METABOLISM OF TRICHLOROETHYLENE IN THREE SPECIES: RAT, MOUSE, AND HUMAN

C.M. Garrett, D.A. Mahle, and J.C. Lipscomb
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NEW CONTINUOUS BREATH MONITORING SYSTEM TO MEASURE EXPOSURE TO TOXIC VOLATILE ORGANIC COMPOUNDS

S.M. Gordon, P.J. Callahan, and D.V. Kenny
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PREIMPLANTATION EFFECTS OF AMMONIUM DINITRAMIDE (ADN) ADMINISTERED IN THE DRINKING WATER OF SPRAGUE-DAWLEY RATS

L.J. Graeter, R.E. Wolfe, E.R. Kinkead, C.D. Flemming, and J.R. Cooper
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DETERMINATION OF METABOLIC CONSTANTS FOR TRICHLOROETHYLENE IN THE B6C3F1 MOUSE FROM GAS UPTAKE STUDIES

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A DOSE-DEPENDENT WEIGHT OF EVIDENCE APPROACH FOR TOXICOLOGIC INTERACTIONS

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COMBINING THE RISK ASSESSMENT AND PUBLIC HEALTH ASSESSMENT METHODOLOGIES TO ESTIMATE HEALTH EFFECTS AND DETERMINE AREAS FOR ECOLOGICAL AND ENVIRONMENTAL IMPROVEMENT

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STRATUM CORNEUM PERMEABILITY COEFFICIENT DETERMINATION FOR VOLATILE CHEMICALS USING THERMAL GRAVIMETRIC ANALYSIS METHODS

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ESTIMATING ECOLOGICAL RISK TO TERRESTRIAL RECEPTORS AT J-FIELD, ABERDEEN PROVING GROUND, MARYLAND

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REAL-TIME ANALYSIS OF EXPIRED RAT BREATH USING TANDEM MASS SPECTROMETRY FOR CARBON TETRACHLORIDE AND ITS METABOLITES

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SEX AND SPECIES DIFFERENCES IN GSH-DEPENDENT TOXICITY AND METABOLISM OF TRICHLOROETHYLENE (TRI) AND PERCHLOROETHYLENE (PER)

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CYTOCHROME P-450 AND GLUTATHIONE S-TRANSFERASE DEPENDENT TRICHLOROETHYLENE METABOLISM IN HUMAN HEPATOCYTES

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TISSUE DISTRIBUTION OF TRICHLOROACETATE IN B6C3F1 MICE AND FISCHER 344 RATS

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MATHEMATICAL MODELING OF SKIN DIFFUSION CELLS

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IN VIVO DERMAL ABSORPTION OF DICHLOROBENZENE, CHLOROPENTAFLUOROBENZENE AND TRIDECAFLUOROiodohexane IN FISCHER 344 RATS

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CHARACTERIZATION OF PCB-CONTAINING MATERIALS ON U.S. NAVAL SUBMARINES

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ECOLOGICAL RISK ASSESSMENT PROGRAM FOR CAPE CANAVERAL AIR STATION AND PATRICK AIR FORCE BASE

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EXPRESSION OF PROTOONCOGENES, TGF- α AND β IN THE LIVER OF B6C3F1 MICE TREATED WITH TRICHLOROETHYLENE, DICHLOROACETIC AND TRICHLOROACETIC ACID

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ECOLOGICAL RISK ASSESSMENT OF MILITARY FOG OIL OBSCURANT ON THREATENED AND ENDANGERED SPECIES

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EXPERIMENTAL PARAMETERS TO SUPPORT A PHARMACODYNAMIC MODEL FOR ETHANE EXHALATION

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LONG-TERM EFFECTS OF ¹³⁷Cs γ -RAYS ON INDUCTION OF CONGENITAL ANOMALIES IN RATS

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EFFECT OF CHRONIC LOW LEVEL EXPOSURE TO JET FUEL IN POSTURAL BALANCE OF U.S. AIR FORCE PERSONNEL

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PROPOSED BIOLOGICAL MARKERS OF EXPOSURE, EFFECT, AND SUSCEPTIBILITY TO HYDRAZINES

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CHANGES IN INSULIN RECEPTOR BINDING AS A BIOMARKER

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QUANTITATION OF FREE RADICALS IN B6C3F1 MOUSE LIVER SLICES ON EXPOSURE TO FOUR CHEMICAL CARCINOGENS: AN EPR/SPIN TRAPPING STUDY

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ORAL BIOAVAILABILITY OF TPH AND OTHER CHEMICALS IN SOIL: LITERATURE REVIEW OF EXPERIMENTAL ISSUES AND RISK ASSESSMENT APPLICATIONS

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CONTINUOUS EXPOSURE MONITORING TO IMPROVE HUMAN HEALTH RISK ASSESSMENT

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A STATISTICAL METHOD FOR DETECTING DEPARTURES FROM ADDITIVITY FOR USE IN ESTIMATING TOXIC INTERACTION EFFECTS ASSOCIATED WITH EXPOSURES TO TOLUENE AND BENZENE MIXTURES

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REGRESSION EQUATIONS TO ESTIMATE PROVISIONAL TLV/WEEL EQUIVALENT FOR NON-CARCINOGEN CHEMICALS HAVING NO TLV OR WEEL

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USE OF PROBABILISTIC RISK ASSESSMENT AT U.S. AIR FORCE INSTALLATIONS

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DOSE (AND TIME DEPENDENT) BLOCKADE OF PREGNANCY IN SPRAGUE-DAWLEY RATS ADMINISTERED AMMONIUM DINITRAMIDE IN DRINKING WATER

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REPRODUCTIVE SCREEN OF MODULAR ARTILLERY CHARGE SYSTEM ADMINISTERED IN THE DIET OF SPRAGUE-DAWLEY RATS

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DEVELOPMENT OF THE RESRAD FAMILY OF CODES FOR ENVIRONMENTAL RISK ASSESSMENT

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**PHARMACOKINETICS AND PHARMACODYNAMICS OF 3,3',4,4'-TETRACHLOROBIPHENYL
IN THE RATS: PBPK MODEL DEVELOPMENT**

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DEVELOPMENT OF A PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODEL FOR TRICHLOROETHYLENE AND ITS METABOLITES IN B6C3F1 MICE

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In recent years, three carcinogenicity bioassays have indicated that the daily oral administration of TCE in corn oil lead to an increased incidence of hepatocellular carcinoma in B6C3F1 mice. To describe and predict the kinetics of TCE absorption, blood and tissue distribution, metabolism and excretion, and better understand its toxicity, a PBPK model was developed for TCE and its metabolites in mice. This model provides a detailed description of TCE kinetics that combined with relatively simple sub-models for metabolites. The model structure consisted of liver, kidney, lung, richly perfused tissues, slowly perfused tissues and fat that were interconnected by arterial and venous blood pools. Partition coefficients (PCs) for TCE were determined by vial equilibrium method, and PCs of non-volatile metabolites were determined using the methods of Jepson et al. (1994). B6C3F1 mice were given bolus oral doses of 300, 600, 1200, and 2000 mg/kg TCE dissolved in corn oil. At various time points, mice were sacrificed and blood, liver, kidney, lung, and fat were collected. The blood and tissue samples were assayed for TCE and its metabolites, chloral hydrate (CH), trichloroethanol (TCOH), trichloroethanol glucuronide (TCOG), trichloroacetic acid (TCA), and dichloroacetic acid (DCA). This study demonstrated that DCA was formed in mice and its formation and kinetics appeared to be driven by TCA concentration.

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OXIDATIVE STRESS AND PROGRAMMED CELL DEATH OF CULTURED J774A.1 MACROPHAGE CELLS IN ASSOCIATION WITH CADMIUM AND CHROMIUM IONS

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Sodium dichromate (Cr VI) and cadmium chloride (Cd II) are known to induce cytotoxicity and mutagenesis. This *in vitro* study was designed to focus upon toxic and apoptotic potential of these cations on cultured J774A.1 cells. These cells were incubated with 0.20, 0.40, and 0.60 μ M concentrations of these cations for 0, 24 or 48 hrs at 37°C. Cr VI- or Cd II-induced changes in cell morphology were detected using phase contrast microscopy. The overall intracellular oxidized states of cells were measured at an excitation wavelength of 513 nm by Laser Scanning Confocal Microscopy using 2,7-dichlorofluorescein diacetate (DCFDA) as the probe. Concentration dependent increases in fluorescence intensity were observed. Signal was quantitated by integrating fluorescence over a user defined area cell number. Approximately 3.6-8.2 fold increase in fluorescence intensity were observed following treatment with Cr VI or Cd II ions. Concentration- and time-dependent influences of Cr VI and Cd II ions on succinate dehydrogenase, a marker of mitochondrial electron transport chain, were determined using MTT assay. Significant increase in enzyme activity was observed with both these ions at 0.40- and 0.60 μ M concentrations at 24 hrs, while a significant decrease in enzyme activity was observed at 48 hrs. Cr VI produced a more pronounced effect as compared to Cd II ions. Fragmentation of nuclear DNA is a biochemical hallmark of programmed cell death (apoptosis), which was assessed using TUNNEL (TdT-mediated dUTP-biotin nick end labelling) method employing fluorescent microscopy. Concentration- and time-

dependent induction of apoptosis was observed with both cations. Cr VI was shown to induce more toxic effects at lower concentrations than Cd II ions. These results clearly indicate that both cations are toxic, producing oxidative tissue damage and apoptosis.



ASSESSING HEALTH RISK ASSOCIATED WITH INDIRECT EXPOSURE TO COMBUSTOR EMISSIONS: RE-EVALUATED

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In 1990, the U.S. Environmental Protection Agency (EPA) developed "Methodology for Assessing Health Risks Associated With Indirect Exposure to Combustor Emissions: Interim Final (EPA/600/6-90/003)". This descriptive methodology was designed for broad application to numerous emitted pollutants (multi-pollutant) and different types of stationary combustion sources.

The organization of the Indirect Exposure (IE) methodology document reflects the four-step process of risk assessment: hazard identification, dose-response assessment, exposure assessment, and risk characterization. However, most of the emphasis is placed on developing a set of algorithms for estimating human exposures resulting from the fate and transport of emitted pollutants transferred from the atmosphere to environmental media and biota.

The original methodology has been regarded as a first step in moving the U.S. EPA beyond the analysis of one medium (air) to the evaluation of other media and exposure pathways. The next step of this process includes making revisions to the 1990 methodology based on a recent Agency-wide

review of the methodology. For example, a proposed change to the existing methodology involves the inclusion of the human breast milk pathway.

This poster presentation describes the original methodology and the proposed revisions to the original methodology.



ECOLOGICAL RISK ASSESSMENT OF A HAZARDOUS WASTE SITE IN THE MOJAVE DESERT OF CALIFORNIA

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An assessment of the potential effects of hazardous wastes on ecological receptors at a site in the western Mojave Desert was conducted. A risk assessment approach combining guidance from the U.S. EPA Risk Assessment Forum, U.S. EPA Region IX, and California Department of Toxic Substance Control was followed. An exposure assessment identified those pathways most likely to contribute to risk of adverse effects to the representative species. The exposure model included uptake of soil chemicals of potential ecological concern (COPECs) by xerophytic plants and other standard exposure pathways used in food chain modeling. In addition, we developed and applied new models for predicting exposure of representative species to COPECs via pathways that are frequently ignored in risk assessments but that we suspected to be significant sources of exposure at this site. Uptake of contaminants in groundwater was modeled for phreatophytic plants by applying bioconcentration factors developed to predict transfer from soil-to-plants to transfer from groundwater-to-plants. Inhalation of contaminated burrow air by burrowing mammals was modeled by 1) combining the Farmer and Huang-Falco surface emission models to calculate concentrations of VOCs in subsurface soil gas based on concentrations in subsurface soil and 2)

using species-specific exposure factors (e.g., inhalation rate and daily time spent in burrows) to estimate intake. For each representative species, the predicted intake from all pathways was compared to a reference toxicity value (RTV) developed from toxicity studies found in the open literature. RTVs were derived from reproductive and/or survival studies for animal species and physiological effects for plant species. The ratio of predicted intake to the RTV produced a Hazard Quotient (HQ). HQ values that exceeded 1 were evaluated further to characterize potential ecological risk by assessing the degree of uncertainty associated with the HQ and signs of stress upon the ecological receptor. Although some uncertainty was associated with the new exposure models, they helped to provide a more comprehensive prediction of exposure and to locate areas where cleanup or further study was warranted.



COMPUTER-AIDED DESCRIPTION OF CHEMICALLY INITIATED OXIDATIVE STRESS *IN VITRO*

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A biologically based pharmacodynamic (BBPD) model based on a description by Vroegop et al. (Free Rad. Biol. Med. 18: 141, 1995) was developed to simulate the dose-dependent biological effects of chemically induced oxidative stress in tissue preparation *in vitro*. The model was written in Advanced Continuous Simulation Language (ACSL) with a FORTRAN sub-routine, and simulations were performed using SIMUSOLV software with optimization capabilities (Dow Chemical Co., Midland, MI) on a VAX/VMS mainframe computer. The BBPD model simulated formation of free radicals over time as a function of the prooxidant chemical concentration, and predicted the dose-dependent response of cellular receptors at each time. The BBPD model was calibrated with the literature data

for free radical generation in liver slices (Steel-Goodwin et al., Toxicologist 15: 30, 1995), and for dose-dependent effects of oxidative stress in cultured neuronal N 18 hybridoma cells (Vroegop et al., 1995). The BBPD model allowed us to distinguish between the "one-hit" targeted mode of action of free radicals and the "multi-hit" stochastic interaction with multiple nonspecific cellular targets. It is suggested that the algorithm developed and calibrated with experimental data *in vitro* may be employed for future dose-response characterization of action of prooxidant chemicals *in vivo*, using the physiologically based pharmacokinetic/dynamic models.

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DETERMINATION OF BLOOD:AIR PARTITION COEFFICIENTS FOR POORLY SOLUBLE CHEMICALS

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Due to the difficulty in measuring partition coefficients for poorly soluble chemicals in blood, an alternate to the well-established vial equilibration method was devised. In the vial equilibration method, chemical headspace concentrations in reference vials and test vials were compared to determine the blood:air partition coefficient. When the resulting blood:air partition coefficient was less than one, often these values were compromised by large standard deviations. This phenomenon is true for Halon 1301 and some of its potential replacements. In the method described here, a system was assembled in order to obtain a more direct measurement. Approximately eight milliliters of heparinized rat blood was placed into an exposure flask and exposed to vapor of the chemical of interest at a rate of 30 ml/min. Microdialysis probes were used to determine when the system reached equilibrium. Every

30 minutes, the exposure concentration was analyzed three times by gas chromatography. Once the system attained equilibrium, nine 50 μ l blood samples were drawn and placed into 12 ml headspace autosampler vials. The vials were heated at 55°C for at least 30 minutes before the headspace was analyzed by hand injections on a gas chromatograph. All chemicals studied had a boiling point less than 50°C to ensure that all of the chemical was driven into the headspace. The blood:air partition coefficients were calculated from the blood headspace concentration and the exposure concentration data. Results indicate that this modified version of the vial equilibration technique yields suitable blood:air partition coefficients for chemicals that are poorly soluble in blood.



DETAILED SOIL SURVEY IN ECOLOGICAL RISK ASSESSMENT: LOCATING BACKGROUND SOIL SAMPLES TO REDUCE UNCERTAINTY AND COST

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In terrestrial ecosystems, soils are the repositories for chemically persistent pollutants in the environment. By their nature most soils tend to sorb many such pollutants, reducing acute toxicity. But ultimately through buffer action, soils also become the media-source for a reduced rate of entry of such pollutants into the food chain, and ultimately the focus for possible ecological risk assessment (ERA) and remediation efforts. Detailed soil surveys have been prepared for most counties throughout the USA by soil scientists. Unfortunately soil surveys as field resources for ERA have frequently been overlooked, although detailed soil surveys contain a wealth of information useful to ERA in support of BRAC and IR. Detailed soil surveys locate, identify, and define soil types/phases/associations on

aerial photographic maps by mapping symbols, and in textual descriptions of soil properties and profiles. Detailed soil surveys are invaluable tools for locating and identifying soil types with similar properties, as are needed for background/comparison samples to those in polluted areas. Furthermore, modern soil surveys include additional information on soil physiography, relief, drainage, and geology; weather/climate; land uses and resources. Use of detailed soil surveys in ERA helps locate appropriate background/comparison soil samples, thus reducing overall cost, and uncertainty in ERA and soil-remediation decision making.



UPTAKE AND PHYTOPHYSIOLOGICAL RESPONSE OF CROP SPECIES TO IRRIGATION WATERS CONTAINING LOW CONCENTRATIONS OF RDX AND TNT

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Crop species grown in site-collected soil were irrigated with water containing cyclotrimethylenetrinitramine (RDX) and 2,4,6-trinitrotoluene (TNT) to simulate groundwater contamination and field conditions at Cornhusker Army Ammunition Plant (CAAP), NE. This is the first investigation of plant response to low levels of RDX and TNT in irrigation waters. Pots were watered to soil water-holding capacity (WHC) throughout the life-cycle of each species in an environment-controlled greenhouse. Irrigation water treatments were 2, 20, and 100 ppb RDX; 2, 100, 800 ppb TNT; 100 ppb RDX + 800 ppb TNT; or uncontaminated water. The relative order of soil loading of RDX and TNT in response to evapotranspirative demand was tomato > alfalfa = corn = soybean > bush bean > lettuce > radish. Uptake of RDX in alfalfa shoots, corn stover, and lettuce leaves was positively correlated with treatment level.

Concentrations of RDX and TNT in the edible portions of these field crop and home garden plant species were determined, and were generally below or equal to soil loading concentrations with no bioconcentration of RDX or TNT. RDX was not significantly ($p=0.05$) taken up into tomato fruit, soybean seed, bush bean fruit (seed and pod), or radish root. TNT was not significantly ($p=0.05$) taken up into the edible tissues of any of the crops in this study. Yield and biomass of tomato fruit, corn stover, soybean seed, and bush bean fruit were significantly ($p=0.05$) less when irrigated with the RDX+TNT treatment compared to controls. Alfalfa shoot, lettuce leaf, and radish root yield and biomass were unaffected by treatment level. Results from this study will be discussed relative to site-specific criteria for CAAP and to previous uptake studies.



SELECTED APPLICATIONS OF REDUCED UNCERTAINTY FACTORS IN NONCANCER RISK ASSESSMENT

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Guidelines have been developed with the U.S. Environmental Protection Agency for applying uncertainty factor to noncancer risk assessment. In many recent cases reduced values for uncertainty factor of less than ten have been used. The specific circumstances that justify reduced uncertainty are: partial definition of the sensitive subpopulation among humans, partial database limitations, use of a minimal LOAEL, risk assessment for an essential nutrient, and risk assessments based upon studies in nonhuman primates. Details of the rationale for each of these circumstances will be provided.



TRICHLOROETHYLENE METABOLISM IN HEPATIC MICROSOMAL AND S₉ PROTEIN OF THE JAPANESE MEDAKA

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Trichloroethylene (TRI) is a common groundwater contaminant that has been shown to be tumorigenic and toxic in laboratory animals. The toxicity of TRI appears to be contingent upon the production of cytochrome P-450-dependent metabolites. Cytochrome P-450 2E1 metabolizes TRI in mammals; however, this isoform of cytochrome P-450 has not been reported to be expressed in the fish species examined to date. The objective of this study was to determine whether the Japanese medaka minnow (*Oryzias latipes*) metabolizes TRI to chloral hydrate (CH) and trichloroethanol (TCOH) in a manner similar to rats, mice, and humans, thereby supporting the role of the medaka in risk assessments for TRI.

Livers were removed from euthanized male and female medaka and prepared by standard methods. The protein content of samples was determined using the BCA method and microsomal P-450 content was determined by carbon monoxide binding. Protein recovery data indicate a gender-specific distribution of total liver mass, total liver protein, and S₉ (metabolically active) protein. Although the body mass of the adult female is approximately 71% that of the male, the female has more liver mass (18.93 mg; 5.35% body mass) per fish than the male (12.44 mg; 3.27% body mass), more S₉ protein (1.54 versus 0.88 mg per gram body mass), more cytochrome P-450 (0.38 versus 0.27 nmoles P-450 per gram body mass) and more activity towards an enzyme-marker substrate (ethoxyresorufin o-deethylase: 4.734 versus 3.674 pmoles/minute/gram liver). Activity towards dimethylnitrosamine (DMN), a marker for P-450 2E1 activity, was not detectable.

Medaka microsomal protein was exposed to TRI and extracted with ethyl acetate. The extracts were analyzed using gas chromatography (liquid injection) with an electron capture detector and separately using gas chromatography-mass spectrometry. We observed and confirmed the microsomal mediated metabolism of TRI to CH, a precursor of toxic metabolites. Linear relationships between the formation of CH and both the exposure time and the protein concentration were demonstrated. In addition, medaka S₉ protein containing cytochrome P-450 and soluble enzymes was exposed to CH. Incubations were subjected to ethyl acetate extraction and a second method involving acidification and derivitization with dimethylsulfate followed by hexane extraction. Ethyl acetate and hexane extracts were analyzed by gas chromatography (liquid injection) with an electron capture detector. Both methods demonstrated the metabolism of CH to TCOH, although initial efforts failed to demonstrate trichloroacetic acid formation.

In a second series of experiments, samples of male and female microsomes were analyzed for individual cytochrome P-450 forms via polyacrylamide gel electrophoresis and western immunoblotting with antibodies selective for individual cytochrome P-450 forms. These experiments confirmed expression of the cytochrome P-450 1A isoforms in the medaka, while cytochromes P-450 of the 3A and 2E families were not detected.

Together, these results are the first to indicate that medaka are capable of metabolizing TRI to CH and CH to TCOH. TRI metabolism in a species devoid of characteristic P-450 2E1 (DMN) activity and in which 2E1 protein is not immunologically detectable strongly suggests that TRI can be and is metabolized by P-450 forms other than 2E1. These data further support the medaka's use in environmental and

conventional risk assessments for this particular groundwater contaminant.

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IN VITRO EFFECTS OF AMMONIUM DINITRAMIDE

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Ammonium Dinitramide (ADN) is a high energy compound under study as a replacement for current rocket propellants. This study determined the basic *in vitro* cytotoxicity, stress gene induction, and genotoxicity of ADN. First, liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured to estimate membrane integrity of WB 344 hepatocytes. ADN increased leakage of both these enzymes at all concentrations studied. Normalized EC₅₀s were determined as percentage of controls: ALT EC₅₀ = 2.7mM and AST EC₅₀ = 3.2mM. Next, to measure interactions of ADN with cellular regulatory transcription factors, genetically engineered human cell lines with fused human and bacterial stress-inducible genes were observed for the stress gene induction followed by ADN treatment. The stress reporter gene induction profile reflected that ADN induced the promoter sequences for all genes observed in the assay. Finally, assays to determine genotoxicity/mutagenicity of ADN were performed. These studies measured ADN's capability of damaging DNA, potentially giving rise to mutations and subsequent tumors. ADN exposed yeast cells indicate that ADN has potential for directly affecting intrachromosomal recombination. This study is a vital part of the initial phase of toxicity testing and evaluation of ADN.

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EVOLUTION OF SCIENCE-BASED UNCERTAINTY FACTORS IN NONCANCER RISK ASSESSMENT

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We review the use of uncertainty factors in risk assessment approaches for noncancer health effects, show data that underlie specific areas of scientific uncertainty, and provide illustrative case studies from regulatory agencies of uncertainty factors that are other-than-default values of 10-fold.

We discuss that "safe" doses estimated with standard methods by agencies around the world, whether ADIs, ECNCs, MRLs, TDCs, TCs, TIs, RfCs, or RfDs, should be considered as accurate---but imprecise---estimations of doses or concentrations believed to be without risk to populations of humans (including sensitive subgroups). Restated, these "safe" doses are thought or assumed to be below population thresholds for adverse effect, but the degree to which they underestimate the population threshold is generally not known. We also discuss three novel approaches for variable uncertainty factors that likewise result in estimations of subthreshold doses, but attempt to quantify the degree to which the estimate is below the population threshold.

We show that the science behind the use of uncertainty factors has progressed considerably through a review of papers that highlight available data for each of several areas of uncertainty. Increased knowledge of inter- and intraspecies sensitivity, mechanisms of action, and detailed evaluation of databases have led to modifications employing variable and data-derived uncertainty factors. Variable or data-derived uncertainty factors allow for the incorporation of more scientific data into the assessment which permit the use of factors

other than the standard default values of 10-fold.

We also show original research and case studies drawn from a larger sample of U.S. EPA and Health Canada risk values where uncertainty factors other than a default value of 10-fold were used in the estimation of a RfD, RfC, TDI, or TC. Percentages for the use of these "data-derived" factors vary between 3.6% and 47%, based on either the availability of specific data within that area of uncertainty, by knowledge of the chemical's mechanism of toxic action, by a combination of both, and/or by informed professional judgment. In the case studies, we explicitly review the types of data that have been used to support a change in the default value, why the data support a different UF, and what assumptions have been satisfied or replaced or how the uncertainty was reduced.

We conclude that other than default uncertainty factors are justified when adequate data exist. However, this conclusion is not surprising. Health agencies generally recognized that default values currently used by risk assessors are somewhat protective from the standpoint of the behavior of the "average" chemical, and may in fact be overly conservative based on new data being generated and analyzed. As a result, these agencies are using other-than-default uncertainty factors on a more regular basis. We hope this text encourages the continued use of other-than-default, or data-derived, uncertainty factors by risk assessors whenever sufficient data are available, and fosters better research into noncancer dose response assessment.



PREPARATION OF BOVINE TESTICULAR SLICES: EVALUATION OF TRINITROBENZENE TOXICITY

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A new animal model to evaluate testicular toxicity has been developed and has been used to assess the effects of 1,3,5-trinitrobenzene (TNB). Preparation of slices of testes was successful using tissue obtained from bulls killed for meat processing purposes. This model is noteworthy because it is a novel application of tissue slice technology, it uses tissues which normally would be discarded, and does not require the use of laboratory animals. Testicular cell relationships (interstitial cell and seminiferous tubules) were maintained and slices remained viable for more than 24 hrs, as measured by histopathological examination, cellular enzyme (AST) and potassium leakage, and the rate of protein synthesis. The level of toxicity observed for TNB during a 24-hr incubation was proportional to the dose. Protein synthesis was the most sensitive indicator of toxicity from the standpoint of earliest and most pronounced response. A dose-dependent toxicity was also observed for release of intracellular potassium. As compared to control values, protein synthesis was inhibited throughout the incubation period at a concentration of 100 μ M TNB, whereas inhibition was detected only after 24 hrs at a concentration of 10 μ M. Protein synthesis was completely stopped by TNB (1000 μ M) and the positive control cyclohexamide (100 μ M). Based on responses for all endpoints, TNB, at equimolar concentration, was a more potent testicular toxicant than other toxicants evaluated [ethane-1,2-dimethanesulfonate (4.6 mM) and cadmium acetate (1000 μ M)]. Lipid peroxidation, as measured by formation of thiobarbituric acid reactive substances (TBARS), was high in control slices. TNB prevented the release of TBARS in a dose/response fashion. An explanation of this finding is provided. This model offers a novel approach for studying male reproductive toxicity and reduces experimental cost, and the use of laboratory animals.

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SEVEN DAY DOSE RANGE FINDING STUDIES FOR TOXICITY ASSESSMENT OF LONG CHAIN PETROLEUM HYDROCARBONS

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Contamination of soil and groundwater with petroleum products is a common environmental problem at DOD installations. Remediation of contaminated sites is currently based on risk assessments derived from a single numerical standard for total petroleum hydrocarbons. Since various petroleum products have different toxicity and leaching characteristics, and weathered sites primarily contain long chain hydrocarbons (LCHs), an initiative is under way to establish toxicity parameters for surrogate LCHs. The toxicity parameters can then be used to more accurately assess the risk associated with weathered petroleum-contaminated sites. Several seven-day range-finding studies were performed as the initial step in establishing NOAELs, which will be used to calculate toxicity parameters. Female Fischer 344 rats and male C57BL/6 mice were dosed orally (gavage) with a surrogate LCH, positive control, soil, or control water for seven days. Neurobehavior parameters including open-field activity, grip strength, and auditory startle response, were tested prior to and at the conclusion of exposure. Cage-side observations, body weights, food consumption, gross necropsy, and organ weights were evaluated for toxic effects. Effect and no-effect dose levels were established. N-nonane and n-hexadecane were tested as surrogates of the C9 to C18 LCH group. 2,5-Hexanedione and acrylamide were included as positive neurobehavior controls. An oral (gavage) sterile soil study was performed to examine the feasibility of dosing with contaminated soil. Noteworthy findings in the n-nonane study included perianal irritation and decreased body weights in rats at 3.6g/kg, increased relative (to body weight) liver weight in mice at 1.8g/kg, and increased

relative spleen weight in mice at 3.6g/kg. Noteworthy findings in the n-hexadecane study included lethargy, suppressed weight gain, and increased relative liver, kidney, spleen, and adrenal weights in rats at 3.9g/kg, perianal irritation and swelling in rats at 1.9g/kg, and lethargy and alopecia in mice at 3.9g/kg.



A BIOLOGICALLY BASED KINETIC MODEL FOR THE ISOLATED PERFUSED RAT LIVER

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The isolated perfused rat liver (IPRL) preparation is a useful tool to investigate the role of the liver in the kinetics of xenobiotics in mammals. This model system is particularly useful because it maintains the liver architecture, as well as the normal route of perfusion. In addition, mechanisms of biliary excretion can be investigated. In order to describe the kinetic behavior of xenobiotics in the system, a generic biologically based kinetic (BBK) model for the IPRL system has been coded in ACSL11 and an executable program debugged. The model is designed to take into account the following biochemical and physiological processes in the liver: (1) membrane transport, (2) protein binding, (3) metabolism, and (4) biliary excretion. The model corrects for the removal of samples from the system during the course of the experiment. The current version describes the behavior of both the parent compound and one metabolite, assuming that metabolism can be described by a Michaelis Menten type equation. Additional metabolic pathways can be incorporated into the model as needed. This model can be used to analyze kinetic data for the concentration of the parent chemical and the metabolite in the perfusion medium, in bile, and in the liver at the end of the perfusion.



COMPARISON OF THE *IN VITRO* METABOLISM OF TRICHLOROETHYLENE IN THREE SPECIES: RAT, MOUSE, AND HUMAN

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Trichloroethylene (TRI), a widely used industrial degreasing solvent, has become a common groundwater contaminant, thereby presenting the potential risk of human exposure. TRI is metabolized to chloral hydrate (CH), trichloroacetate (TCA), trichloroethanol (TCOH), and other compounds. Some rodents have developed hepatocellular carcinomas following exposure to TRI and/or its metabolites. However, to date epidemiologists have been unable to demonstrate a link between known TRI exposure and toxicity in humans. Therefore, as part of an ongoing investigation into the toxicities associated with TRI exposure, the kinetics of TRI metabolism in the hepatic microsomes of three species were determined. CH and TCOH formation from TRI at pH 7.4 was evaluated by incubating rat, mouse, and human hepatic microsomes with limiting concentrations of TRI at 37°C for 30 min. TRI was dissolved in acetone and spiked into the incubation mixture at 0.1% per final volume. Although acetone is known to alter the activity of cytochrome P-450IIE1, the enzyme thought to be responsible for the conversion of TRI to CH, it was experimentally shown to have no effect on 2E1 activity at this level. The kinetics for microsomal TRI metabolism indicated K_m values of 55.5, 24.6, and 35.4 μ M and V_{max} values of 1206, 360, and 1356 nmol/min/mg protein, respectively, for the rat, human, and mouse. Trichloroethanol was also formed from TRI in very small amounts. These data indicate that the kinetics of TRI metabolism may differ significantly depending upon the species evaluated, and care should be used when extrapolating rodent data for human risk assessment. Human *in vitro* studies like the ones conducted here are an invaluable step toward the accurate characterization of

the biochemical pathways involved in human metabolism of TRI.

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NEW CONTINUOUS BREATH MONITORING SYSTEM TO MEASURE EXPOSURE TO TOXIC VOLATILE ORGANIC COMPOUNDS

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Analysis of human exhaled breath for toxic volatile organic compounds (VOCs) is well-established in occupational medicine. Its use in environmental applications is relatively recent and not nearly as widespread. Nevertheless, a number of important discoveries have been made in this area, and its value as a means of relating exposure to dose has been steadily increasing.

Most previous efforts to measure trace VOCs in breath have relied on conventional batch methods, in which a sample is first collected in a suitable container before it is transferred to a laboratory for analysis. These techniques typically only provide time-integrated information, and sample collection times are too slow to evaluate exposures due to rapidly changing concentrations.

We have developed new monitoring technology that can provide continuous real-time measurements of trace VOCs in breath and air. The technology makes use of a compact direct air sampling mass spectrometric (MS) system, based on novel ion trap technology. The analytical device is the Teledyne 3DQ Discovery™ quadrupole ion trap mass spectrometer (ITMS), which minimizes the effects due to space charging normally associated with ion traps by the application of filtered noise fields (FNF). The instrument is small and lightweight, and provides a unique means of simultaneously isolating individual target compounds in

complex mixtures. It is, therefore, capable of true selective ion monitoring and, when operated in the MS/MS mode, permits unambiguous characterization of many compounds of environmental interest with high sensitivity and specificity.

We have used the device with two direct air sampling (DAS) interfaces (semipermeable tubular membrane; atmospheric sampling glow discharge ionization source) and a new breath collection system to measure select VOCs at trace levels. The main advantage of the method lies in its ability to generate large quantities of data to follow the uptake, distribution in the body, and elimination of VOCs. Typically, in a brief sampling session, more data points can be collected, especially during the crucial first minutes immediately following initiation or cessation of exposure, than in a full-scale chamber study using the standard integrated sampling methods - and at less cost. This presentation will describe the performance of the breath inlet/DAS/ITMS combination and describe its use in several pilot studies of environmental exposures to VOCs.

Although the research described in this document has been funded wholly or in part by the United States Environmental Protection Agency through Cooperative Agreement CR 822062-01-0 to Battelle Memorial Institute, it has not been subjected to Agency review and therefore does not necessarily reflect the views of the Agency, and no official endorsement should be inferred.



PREIMPLANTATION EFFECTS OF AMMONIUM DINITRAMIDE (ADN) ADMINISTERED IN THE DRINKING WATER OF SPRAGUE-DAWLEY RATS

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The Department of Defense is evaluating ammonium dinitramide for use as a solid

rocket propellant and as an explosive. Previous studies have shown that ADN is a reproductive toxicant, causing implantation failure in Sprague-Dawley rats when it is administered during the preimplantation period of gestation. This study was designed to evaluate the implantation failure. After successful mating, female rats were treated with 2.0 grams/liter ADN in their drinking water for 24, 48, 72, or 96 hours before preimplantation embryos were harvested from the oviducts or uterine horns. The stage of development of the embryos was evaluated by phase contrast microscopy. On gestation day one, 10.2 ± 0.48 (SEM) and 10.3 ± 0.42 morphologically normal 2-cell embryos were harvested from the treatment and control groups, respectively. On gestation day two, the development of the embryos harvested from the treated animals was either slowed or halted when compared to the control embryos. By gestation day four, 10.3 ± 0.49 embryos from control animals were harvested from the uterine horns; these had developed to the late morula or blastocyst stage. On gestation day four in the treated group, 7.3 ± 1.4 developmentally halted (4-cells or less) or degenerate embryos were collected from the oviducts (82%) or uterine horns. These data suggest that the implantation loss seen in animals treated with ADN is due to embryo lethality.

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DETERMINATION OF METABOLIC CONSTANTS FOR TRICHLOROETHYLENE IN THE B6C3F1 MOUSE FROM GAS UPTAKE STUDIES

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The metabolic capacity of TCE in male B6C3F1 mice was determined using a gas uptake chamber. A 3-L closed recirculated

gas uptake exposure system containing 3 mice was used to collect a series of uptake curves for eight concentrations of TCE ranging from 100 to 7000 ppm. A physiologically based pharmacokinetic (PBPK) model was used to analyze the kinetic uptake data using SIMUSOLV (Dow Chemical Company) software on an IBM mainframe terminal. The PBPK model for TCE in B6C3F1 mice consisted of six compartments with experimentally derived tissue:blood partition coefficients for fat, liver, and muscle and a blood:air partition coefficient. In order to simulate the uptake profiles for the series of TCE concentrations and to estimate the *in vivo* Michaelis-Menten metabolic constants in B6C3F1 mice, the values of V_{maxc} and K_m were adjusted to obtain the best fit. The ranges of values used were $K_m=0.10-5.0$ mg/L and $V_{maxc}=5.0-40.0$ mg/kg/h. Because this did not provide a good fit to the data, the kinetic parameters were set to those reported in Fisher et al., (1991; $K_m=0.25$ mg/L and $V_{maxc}=32.7$ mg/kg/h. The model failed to predict the initial rapid clearance of TCE for the lower concentrations and to a less degree the higher concentrations. As the exposure progressed, the model overpredicted the clearance of TCE except for the 7000 ppm concentration. Several model parameters were adjusted in order to obtain a better fit. The cardiac output, ventilation rate, and fat content adjustments improved the fit slightly. Further research aimed at quantifying metabolic activity in B6C3F1 mice, such as P-450 activity and 2E1 status, are planned.



A DOSE-DEPENDENT WEIGHT OF EVIDENCE APPROACH FOR TOXICOLOGIC INTERACTIONS

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The dose-additive Hazard Index is the U.S. EPA's recommended approach for

component-based risk assessment of noncancer toxicity of mixtures. This index does not accommodate information on toxicologic interactions (synergism, antagonism). We have developed a replacement: a four-step Weight of Evidence (WOE) approach for an interaction-based Hazard Index. First, a WOE value is calculated for each pair of chemicals in the mixture of concern. This WOE factor reflects the strength and consistency of the available studies regarding potential interaction. Second, a maximum interaction magnitude (M) is determined for each chemical pair (or assigned to a default value). Third, the WOE value is combined with the maximum M and then scaled according to the exposure levels of the components. Last, the "Interaction Hazard Index" is then calculated as the sum of all these scaled factors. This interaction Hazard Index is interpreted as before, i.e., values less than one indicate acceptable exposures.

The scaling is the major improvement over other interaction approaches. It is designed to reflect certain hypotheses about toxicologic interactions: 1) the interaction decreases with dose, resulting in dose addition at low exposures, 2) the binary interaction is largest when the two chemicals are at equitoxic exposure levels, and 3) the higher level interactions, e.g., the impact of Chemical C on the interaction between A and B, is minor compared to the binary interactions. The advantage of this method of accounting for interactions compared to other weight of evidence methods is that it includes several items that can be tested, directly estimated, or replaced by better models as the empirical and mechanistic information improves. In addition, the procedure is easily implemented by a personal computer for application to actual exposures. After acquiring a database of the intrinsic factors, WOE and M, the risk assessor can input the environmental exposure levels and generate the situation-specific interaction Hazard Index.



COMBINING THE RISK ASSESSMENT AND PUBLIC HEALTH ASSESSMENT METHODOLOGIES TO ESTIMATE HEALTH EFFECTS AND DETERMINE AREAS FOR ECOLOGICAL AND ENVIRONMENTAL IMPROVEMENT

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Risk assessment methodology uses actual environmental samples to estimate health effects from sites ranging from industrial work sites to residential areas near hazardous waste sites. A public health assessment characterizes the nature and extent of hazards, identifying communities where public health action is needed. The objective of this study was to use the methodologies of both risk assessment and the public health assessment to estimate health effects for living on an Army installation located on a group of remote Pacific islands. While the sampling performed during this study focused on the extent of existing environmental contamination, public forums and interviews were used to address other public health issues.

This study was different from either the regular risk assessment or the public health assessment because it was not restricted to the guidance of either. The classic risk assessment ignores hazards which are not related to environmental chemicals. The classic public health assessment does not have the luxury of producing its own environmental data.

This procedure also offers a unique quality, because the sampling could not be located around the focal point of a contamination source, and the sampling was designed around a small population and predictable (or at least measurable) patterns of exposure. Sampling was based solely on the exposure patterns of the post's inhabitants. The

inhabitants were observed to determine their habitual patterns and common destinations. The islands were then sub-divided by destination areas and the sampling was randomized within each destination. This is a modification of a randomized block design favored by researchers on the physical environment. The study sub-areas consisted of: Industrial Area, Playgrounds, Softball Spectator Area, Residential Housing, Softball Fields, Retail Area, Golf Course, Beach Area, Multi-use Field, High School, Elementary School, and Running Track.

Risk communication was conducted through a series of public forums. Poster sessions were set up in the retail areas of each island to test the public's habit patterns and to gather information on the resident's actual concerns. These concerns were not limited to the goals and sampling activities of the study. The poster sessions were designed to allow gathering concern for any facet of island life.

The opinions or assertions contained herein are the views of the author(s) and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.



STRATUM CORNEUM PERMEABILITY COEFFICIENT DETERMINATION FOR VOLATILE CHEMICALS USING THERMAL GRAVIMETRIC ANALYSIS METHODS

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The Stratum Corneum (SC) can be the primary barrier to transdermal penetration of volatile organic occupational and environmental chemicals. Much of the quantitative information to describe or compare the movement of chemicals through the SC is contained in the SC permeability coefficient. The SC permeability coefficient

has the units of cm/hr and is a composite of the diffusion coefficient, SC to air partition coefficient and the diffusion pathlength distance. Thermal gravimetric analysis (TGA) of SC during exposure to volatile organic chemicals was used to estimate SC permeability coefficients. Whole thickness human or rat skin was excised and dermatomed to a depth of 0.025 in. The skin was treated with trypsin for 2 hours at 37°C in order to separate the SC from the underlying viable epidermis. The SC was dried and stored in a dessicator. Five milligrams of SC were placed into the titanium sample pan of the TGA cell. The cell was maintained at 32°C and purged with 50 mL/min of air and the weight was monitored until equilibrium was reached. The flow was then stepped to 1.225 L/min and maintained until equilibrium was again reached. Then 10000 ppm of chemical vapor was introduced into the TGA cell at 1.225 L/min. The weight increase per unit time was evaluated using a non steady-state diffusion equation in order to obtain a SC diffusion coefficient and the equilibrium phase was used to obtain a SC to air partition coefficient. The information derived from the TGA combined with SC thickness measurements from microscopic analysis allowed for estimation of SC permeability coefficients. This approach was proven useful for determining volatile organic chemical SC permeability coefficients in different species and under different temperature and exposure conditions.



ESTIMATING ECOLOGICAL RISK TO TERRESTRIAL RECEPTORS AT J-FIELD, ABERDEEN PROVING GROUND, MARYLAND

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Accurately characterizing ecological risk in terrestrial ecosystems has come under increasing criticism. Problems range from

ecological, species-relevant, toxicological data gaps to gross uncertainties in exposure model development. We attempted to reduce these uncertainties through field application of a three-step process: 1) receptor identification; 2) exposure analysis; and 3) measures of reproductive performance. We concentrated on three classes of receptors: birds, amphibians, and small mammals. Our results provided reliable exposure information incorporated into models and supported conclusions through populational relevant data. This research describes these processes, along with the results of these analyses.



REAL-TIME ANALYSIS OF EXPIRED RAT BREATH USING TANDEM MASS SPECTROMETRY FOR CARBON TETRACHLORIDE AND ITS METABOLITES

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Real-time breath analysis offers a non-invasive method to detect exposure to toxic air pollutants. Continuously monitoring breath for a parent toxicant and its metabolite(s) can provide input into physiologically based pharmacokinetic (PBPK) models to describe the biokinetics of a chemical in the body. Real-time analyses are better than batch sampling methods because of the rapidly changing kinetics of elimination of some volatile chemicals from the body.

A Sciex TAGA 6000E MS/MS equipped with the low pressure chemical ionization source (LPCI), along with a Finnigan ITMS equipped with an atmospheric sampling glow discharge ionization source (ASGDI) and the Teledyne HST-1000 accessory kit (for filtered noise field [FNF] operation) were used for these experiments. The expired breath from up to six laboratory rats was introduced separately into two tandem mass spectrometers by way

of a manifold. This device is a modification of a human breath interface patented by Battelle. Carbon tetrachloride, its main metabolite, chloroform, and three markers of lipid peroxidation: ethane, pentane, and acetone, were simultaneously monitored, in real-time, in the expired breath of the rats exposed to carbon tetrachloride. Detection limits for chloroform and its metabolites was on the order of 1 - 10 ppb. The real-time concentration data was then input into a PBPK model to determine target tissue doses. Since the methodology provides information on real-time metabolic processes and toxic responses, the implications for improving health risk assessments are substantial.



SEX AND SPECIES DIFFERENCES IN GSH-DEPENDENT TOXICITY AND METABOLISM OF TRICHLOROETHYLENE (TRI) AND PERCHLOROETHYLENE (PER)

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Sex and species dependent differences exist in the hepatic and renal toxicity of Tri and Per. To assess the role of GSH-dependent metabolism in toxicity of Tri and Per and the utility of *in vitro* animal models in estimating risk of Tri and Per to humans, we: 1) determined rates of metabolism of Tri and Per by GSH conjugation in freshly isolated renal cortical cells and hepatocytes from male and female F344 rats and in hepatic and renal subcellular fractions (cytosol, microsomes) from male and female F344 rats and B6C3F1 mice; 2) determined time and concentration dependence of release of lactate dehydrogenase (LDH) from freshly isolated renal cortical cells and hepatocytes from male and female F344 rats after acute exposures to Tr, Per, or their GSH and/or

cysteine conjugates to assess acute cytotoxicity.

Both Tri and Per undergo GSH conjugation to form S-(1,2-dichlorovinyl)glutathione (DCVG) and S-(1,1,2-trichlorovinyl)glutathione (TCVG), respectively, in cells and subcellular fractions from liver and kidney. Further metabolism in kidney generates reactive species that may produce acute nephrotoxicity or nephrocarcinogenicity. DCVG and TCVG were measured as dinitrophenyl derivatives with ion-exchange HPLC and detection by absorbance at 365 nm. Tri metabolism to DCVG was demonstrated in isolated renal cortical cells from rats and rates were approximately twice as fast in males as in females. However, rates of DCVG formation in renal cells from male rats were only about 10% of those in isolated hepatocytes from male rats. Rates of DCVG formation in renal and hepatic subcellular fractions from male and female mice were generally two to five times those in the corresponding fractions from male and female rats. Rates of Per metabolism to TCVG were similar to those for Tri metabolism to DCVG in corresponding renal cells but were 25 to 50% lower than those for Tri metabolism to DCVG in corresponding liver subcellular fractions. Thus, sex-dependent differences in rates of GSH conjugation in renal and hepatic tissue from rats corresponds to the *in vivo* susceptibility to renal injury, with higher rates of GSH conjugate formation in males correlating with greater toxicity in males. In contrast, male and female mice do not generally exhibit renal injury from Tri and Per, although the rates of GSH conjugation were higher in tissue from mice than from rats.

Acute cytotoxicity of Tri, DCVG, S-(1,2-dichlorovinyl)-L-cysteine (DCVC), Per, and TCVG in renal cortical cells from male rats varied from moderate (30 to 50% LDH release) at high doses (Tri) to marked (70 to 90% LDH release; other compounds). In contrast, Tri, Per, and TCVG were non-toxic

(< 25% LDH release) at concentrations up to 10 mM while DCVG exhibited minimal cytotoxicity (25 to 35% LDH release) at high doses and DCVC exhibited moderate cytotoxicity in renal cortical cells from female rats. Thus, sex-dependent acute cytotoxicity of Tri, Per, and their metabolites in renal cells correlates with their greater nephrotoxicity and nephrocarcinogenicity in male rats *in vivo*. Isolated hepatocytes from male rats exhibited moderate cytotoxicity from Tri and extensive cytotoxicity from Per whereas isolated hepatocytes from female rats exhibited no cytotoxicity from either Tri or Per with concentrations up to 10 mM. This pattern of acute cytotoxicity in isolated hepatocytes, both comparing susceptibility to Tri versus Per and males versus females, corresponds to *in vivo* patterns of hepatic injury.

These studies demonstrate correlations between rates of GSH conjugation and susceptibility to renal injury in male and female rats and mice. The relatively high rates of GSH conjugation in mouse renal tissue with no *in vivo* nephrotoxicity is most likely due to the markedly higher rates of cytochrome P-450-dependent metabolism of Tri and Per in mice as compared to rats.

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CYTOCHROME P-450 AND GLUTATHIONE S-TRANSFERASE DEPENDENT TRICHLOROETHYLENE METABOLISM IN HUMAN HEPATOCYTES

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Trichloroethylene (TRI) is an industrial degreaser and anesthetic agent which is a common groundwater contaminant. Human health risk assessments for TRI are currently based on rodent data. The toxicity associated with TRI exposure in the rodent is produced by TRI metabolites and induction of cytochrome P-450 (P-450) dependent metabolism results in increased TRI toxicity in laboratory animals. Major metabolites of TRI in the rodent and human are trichloroethanol (TCOH) and trichloroacetic acid (TCA). Dichloroacetic acid (DCA) has been found in rodents exposed to TRI, but has not been reported in samples from TRI-exposed humans. TRI is also metabolized through a glutathione S-transferase (GST)-dependent pathway to form dichlorovinyl glutathione (DCVG). It is thought that DCVG is further processed in the kidney to yield the nephrotoxic dichlorovinyl cysteine (DCVC). Subcellular localization studies and substrate structure similarities suggest that GSTs of the theta class conjugate TRI. Theta class GST are expressed in mouse erythrocytes, and in 60% of the human population.

The present study was designed to determine whether human hepatocytes procured from organ donor agencies metabolize TRI via these pathways. Their use would yield data on the metabolic activation of this important environmental toxicant in the species of interest, the human. To avoid degradation of P-450, hepatocytes were evaluated in primary culture within 30 hours of organ removal. Hepatocytes were incubated in Chee's medium for two hours, under 95% O₂/5% CO₂ containing 0 - 10,000 ppm TRI. Toxicity was assessed by AST,

ALT, and LDH release. Cytochrome P-450 dependent TRI metabolism was assessed by quantification of chloral hydrate (CH), trichloroethanol (TCOH), and trichloroacetic acid (TCA); GST-dependent metabolism was assessed by quantification of DCVG.

Metabolites derived from the cytochrome P-450 dependent pathway were approximately one-fourth that derived from the GST-dependent pathway, and TCOH accounted for 95% of the P-450 dependent metabolites. DCA was not identified in the incubations. P-450 dependent metabolism was saturated above concentrations of approximately 1,000 ppm in headspace, while DCVG formation was inhibited at higher concentrations. CH was found in higher concentrations at the earlier time points and declined as incubation time progressed. TCA production proceeded at a steady rate throughout the incubation period. DCVG formation did not proceed appreciably beyond 30 minutes incubation time. Maximal DCVG production in two samples analyzed reached a maximum at 500 ppm TRI and declined sharply above this concentration. Demonstration of glutathione conjugation of TRI in human hepatocytes *in vitro* provides confirmation of this pathway in the human and adds complexity to overall TRI metabolism and competition between GST and P-450 metabolism: theta class GST is also distributed to the blood, the vehicle which distributes inhaled TRI to the liver.

These data demonstrate the use of isolated hepatocytes from human organ donors for use in determining metabolic activation of toxicants. We have demonstrated the activity of Phase I, Phase II, membrane-bound and soluble enzymes in this evaluation. Data from this study can be used in the examination and description of the metabolic pathway of TRI in the human. Data such as these will reduce the requirement for research animals, while improving the quality of data used to predict human risk by eliminating species-to-species extrapolation

of results. For these reasons, the human hepatocyte as procured from organ donor agencies may be well-suited to examine chemical metabolism in human liver.

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TISSUE DISTRIBUTION OF TRICHLOROACETATE IN B6C3F1 MICE AND FISCHER 344 RATS

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Trichloroacetate (TCA) has been chosen as a test chemical for the development of a pharmacokinetic (PBPK) model for water soluble compounds. TCA is formed from the metabolism of some industrial solvents such as trichloroethylene and tetrachloroethylene. It is also a byproduct of water chlorination. Mice dosed with TCA have developed hepatocellular carcinomas while rats have not. It has been shown to cause developmental malformations in rats. Male B6C3F1 mice received a continuous 1 μ L/hr dose of 0.03 and 0.3 mg/ μ L TCA in saline via subcutaneous mini-osmotic pumps. Blood and tissues were taken at 5 days, and TCA concentration was quantified by GC after derivitization. Tissue/blood partitions were determined for several tissues. Liver, muscle, and GI/blood partitions were 0.22, 0.07, and 0.12 for the low dose, respectively. Male F-344 rats were dosed with 10 mg/kg TCA in saline by i.v. injection in the lateral tail vein. Blood and tissues were collected at 0.5, 1, 3, 6, 9, 24, and 48 hr. Total radiolabel was determined in oxidized tissues by scintillation counting. For kidney, lung, skin, testes, muscle, brain, and fat, the % radiolabel (tissue/plasma) remained fairly constant from the 1 hr to the 24 hr time point. Liver, heart, intestine, and stomach % radiolabel increased from 1 hr to 24 hr. There was a decrease in % radiolabel at 24

hr for RBC. To determine the amount of radiolabel that was parent TCA, tissue proteins were precipitated with acetonitrile, and the soluble layer was analyzed by HPLC. A significant fraction of the radiolabel was in the precipitate for liver, indicating the possibility of binding. This description of the tissue distribution of TCA can be used in the development of PBPK model for water soluble compounds.



MATHEMATICAL MODELING OF SKIN DIFFUSION CELLS

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Determining the rate of penetration of chemicals through skin is necessary to assess the hazard with dermal exposure to chemicals in the occupational or environmental setting. Small glass diffusion cells with donor and receptor sides separated by excised skin are commonly used to determine the rate of penetration of chemicals through skin. Flux ($\text{mg cm}^{-2} \text{ hr}^{-1}$) or permeability coefficient (cm hr^{-1}) are common ways to express the amount of chemical which comes through the skin at steady state. One problem with these studies is that steady rates of absorption must be reached in order to accurately calculate flux from the slope of total absorption versus time plot. It is normally necessary to put excess chemical in contact with the skin and wait sufficient time to approximate steady state in order to make flux measurements. The purpose of this investigation was to determine if estimates of the parameters which determine flux could be made prior to achieving steady state conditions. We developed a mathematical description of a static diffusion cell to use as a tool for estimating absorption parameters. Amount of chemical in receptor solution is simulated with the model to provide estimates of flux and diffusion. This tool has been applied to absorption of chemicals through a butyl rubber membrane and

isolated rodent skin. This method of mathematical modeling is useful for getting information from *in vitro* diffusion studies prior to steady state.



IN VIVO DERMAL ABSORPTION OF DICHLOOROBENZENE, CHLOROPENTAFLUOROBENZENE AND TRIDECAFLUOROiodohexane IN FISCHER 344 RATS

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Dermal exposures to chemicals in the workplace may cause a systemic hazard if exposures are prolonged or spread over a large surface area. Dermal absorption kinetics of dichlorobenzene (DCB), chloropentafluorobenzene (CPFB) and tridecafluoroiodohexane (TFIH) were measured in male Fischer 344 rats during carefully controlled dermal exposures to the neat chemical. On the day before exposures the skin was carefully clipped of fur and a small glass cell was superglued to the middle of the rat's back. Blood concentrations were measured serially from an indwelling jugular cannula for up to four hours. Physiologically based pharmacokinetic (PBPK) models for each chemical were used to estimate real-time flux and permeability coefficients for each of these six carbon chemicals. Permeability coefficients varied by over an order of magnitude. As judged by histology, these chemicals all affected the skin to some extent during the exposures. PBPK models suggested that the decrease in blood concentrations during the exposures was due to a decrease in rate of absorption rather than a decrease in concentration on the surface. These studies demonstrate that PBPK models can be used as a tool to understand changes in the absorption process.

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CHARACTERIZATION OF PCB-CONTAINING MATERIALS ON U.S. NAVAL SUBMARINES

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The extent of PCB-containing materials aboard Naval submarines was characterized. A database of submarine media bulk and swipe samples (collected and analyzed between 1990 and 1995) was obtained from the Puget Sound Naval Shipyard (PSNS). The effectiveness of the database for characterizing submarines was considerably limited due to missing descriptors (e.g., material type, location) for many samples. The exclusion of samples for analysis by material category or location may significantly bias the results towards either under- or overestimation. Two types of swipe samples were found in the database, field test kit samples and gas chromatography/electron capture detector (GC/ECD) analyzed swipe samples. Due to considerably lower method detection limits (MDL), the GC/ECD samples were used to characterize bulk and surface contamination.

The different bulk materials sampled included, but were not limited to, mastic, rubber gaskets, aroclor, aramflex, felt insulation, electrical insulation, and paints. A large variance in the sample results was noted among all material types within all compartments of the submarines. As a result, the median concentration value was a better representation of central tendency and potential exposure levels than the mean value. Among the materials containing the greatest concentrations of PCBs are felt, white foam material, and aroclor. The uncertainty of using the data for exposure assessment should be addressed by Monte Carlo analysis.



ECOLOGICAL RISK ASSESSMENT PROGRAM FOR CAPE CANAVERAL AIR STATION AND PATRICK AIR FORCE BASE

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The Ecological Risk Assessment Program (ERAP) conducted basewide Ecological Risk Assessments (ERAs) for Cape Canaveral Air Station (AS) and Patrick Air Force Base (AFB), FL. The ERAP was designed to evaluate potential impacts to ecological receptors related to multi-source chemical releases. Potential effects of individual hazardous waste sites are being investigated under the Installation Restoration Program (IRP). The ERAP supports the IRP.

The basewide ERA consists of four major evaluations: habitat/receptor, geographic/chemical agent, exposure, and hazard. The habitat/receptor evaluation identifies receptors and habitats that may be affected by basewide hazardous waste release. The geographic/chemical agent evaluation geographically delineates chemical concentrations in environmental media, identifies constituents of potential concern (COPCs), and assesses chemical fate and migration pathways. The exposure evaluation identifies receptor-specific total daily intake, primarily through ingestion. The hazard evaluation identifies receptor-specific chemical toxicity based on hazard quotient (HQ) and hazard index (HI) modeling.

ERAP incorporates three major components into the ERA process:

- 1) Basewide representation
- 2) Natural variability
- 3) Geographic depiction

These three aspects are important components of the exposure and hazard evaluations. Basewide exposure and risk were represented using central and maximum media concentrations to develop

average and upper bound total daily intakes, HQs and HIs. Natural variability is accommodated by ERAP with the development of receptor-specific total daily intake and HQ profiles. Profiles are reflective of varying chemical exposure levels and differences in receptor populations with respect to life history parameters and toxicologic susceptibility. Geographic depiction of exposure and hazard were accomplished through Geographic Information System (GIS) mapping. ERAP used GIS to delineate risk levels across spatial gradients using data from multiple sites over relatively large areas.

The exposure and hazard evaluations were separated into two distinct analyses, terrestrial and aquatic food chain models. Each model consists of an exposure and hazard evaluation. The terrestrial exposure and hazard evaluations utilized soil chemical data to delineate regions of similar exposure point concentrations and resultant hazard levels. Basewide exposure and hazard estimates were developed from individual regional values weighted according to percent areal coverage of the total base. Basewide representative total daily intakes and HQs, total daily intake and HQ profiles (reflecting inherent variability) and GIS maps defining soil areas posing risk were produced for the terrestrial foodchain model. Results of the terrestrial model were reflective of past site-related activity.

The ongoing aquatic exposure and hazard evaluations also estimate exposure point concentrations and resultant hazard levels. Identified risks will be associated with critical drainage basins based on chemical monitoring of sediment, surface water, and biota and canal-specific discharge rates. Loadings to the Banana River will assist in identifying critical canals. Canal-specific discharge will be used to weight cumulative basewide risk calculations for aquatic receptors. Basewide representative total daily intakes and HQs, total daily intakes and

HQs, total daily intake and HQ profiles (reflecting inherent variability) and GIS maps depicting critical watersheds posing risk to Banana River receptors will be produced for the aquatic model. Results of the aquatic model will reflect the combined influence of past and ongoing hazardous waste activity at each base.

Overall, ERAP employs an integrated approach designed to identify significant levels of ecological hazard. The use of multifaceted ERA components allows weight-of-evidence interpretation of findings and strength-of-evidence formulation of conclusions.



EXPRESSION OF PROTOONCOGENES, TGF- α AND β IN THE LIVER OF B6C3F1 MICE TREATED WITH TRICHLOROETHYLENE, DICHLOROACETIC, AND TRICHLOROACETIC ACID

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Trichloroethylene (TCE) is an organic solvent used as a metal degreaser which has resulted in measurable quantities found in surface water, groundwater, ambient air, and soil. Its metabolites, dichloroacetic acid (DCA) and trichloroacetic acid (TCA), are found as by-product of chlorinate disinfection. Exposure of humans to TCE, DCA, and TCA is of concern because of the carcinogenic potential. In the present study, we examined the expression of *c-jun*, *c-myc*, and *c-fos* protooncogenes, and TGF- α and β to determine whether these selected protooncogenes and growth factors are deregulated in the process of TCE, DCA, and TCA-induced mouse liver tumors. B6C3F1 female mice were treated with 800 mg/kg TCE or corn oil vehicle via gavage, once daily for 5 days/week, or 3.2 gm/L of DCA or 4.0 gm/L in drinking water. The animals were sacrificed at 1 or 300 days at 45, 90,

and 120 min after the last dose. mRNAs of the protooncogenes were analyzed by Northern blottings and the density calculated by a computerized image analysis system. Proteins of these protooncogenes, and TGF- α and β were detected by immunohistochemistry (BioTek 1000, Santa Barbara, CA). After a single dose or 300 days of 5 days/week exposure of TCE, *c-jun* expression increased from 45-90 min, peaked at 90 min ($p < 0.05$, compared with other time points and control), then decreased by 120 min, and *c-myc* expression was increased as early as 45 min, with a peak expression at 120 min ($p < 0.05$, compared with other time points and control) in the liver, while *c-fos* expression was not detected. TGF- α expression was strongly positive in DCA-induced hepatomas and negative in hepatocytes while TGF- β was strongly positive in hepatocytes and negative in hepatomas. In TCA-treated mice, the expression of TGF- α and β was heterogeneous in liver tumors and hepatocytes. The present results suggest that *c-myc* and *c-jun*, TGF- α and β may play an important role in TCE, DCA, and TCA-induced hepatocarcinogenesis.



ECOLOGICAL RISK ASSESSMENT OF MILITARY FOG OIL OBSCURANT ON THREATENED AND ENDANGERED SPECIES

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Military field training with smokes and obscurants releases compounds into the environment. Different compounds are released to accomplish different scenarios to fulfill a variety of training objectives for military preparedness. Fog oil is one of several obscurants heavily used by the military in training. Fog oil is released using a generator for production of large area screens. Many military installations are also homes for threatened and endangered (T&E)

species, particularly the Red-Cockaded Woodpecker (RCW).

USACERL has completed a preliminary (Tier 1) ecological risk assessment and is collecting additional data to more fully assess potential impacts of fog oil obscurant on T&E species, particularly RCW, at two Army installations in the southeastern region of the United States. The assessment is consistent with the Framework for Ecological Risk Assessment developed by the U.S. Environmental Protection Agency. The assessment includes distribution of the fog oil obscurant in the atmosphere; physical and chemical characteristics of the fog oil; deposition, movement, and fate of fog oil in the environment; and effects of fog oil on organisms of concern, particularly RCW. A mathematical model is used to predict fog oil dispersion and deposition validated by selected field measurements. Movement and fate are predicted based on literature data and quantitative structure-activity relationships (QSAR). Primary environmental removal mechanisms have been identified. Effects are predicted from literature data and laboratory studies. Based on current information, fog oil concentrations and exposure durations known to cause effects are higher and longer than expected exposures of RCW. We are continuing to investigate the potential for chronic effects, behavioral effects, and indirect effects on habitat.

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EXPERIMENTAL PARAMETERS TO SUPPORT A PHARMACODYNAMIC MODEL FOR ETHANE EXHALATION

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A computerized, physiologically based pharmacodynamic (PBPD) model was

developed to simulate the kinetics of ethane generation and disposition in experimental animals. Ethane exhalation is recognized as a reliable index of lipid peroxidation which may be stimulated by pro-oxidant chemicals. The PBPD model was written in Continuous Simulation Language (ACSL), and simulations were performed using SIMUSOLV software on a VAX/VMS mainframe computer. The PBPD model simulated formation of ethane over time, depending on the concentration of the pro-oxidant chemical, and predicted the disposition and exhalation of ethane. The partition coefficients for ethane were determined in mouse tissues *in vitro* and ethane metabolism parameters were measured in mice *in vivo*, using a closed gas uptake chamber. The PBPD model allowed us to simulate the kinetics of ethane exhalation in mice treated with massive doses of either CCl_4 or TCE. It is suggested that the PBPD model may be useful for pharmacodynamic descriptions of oxidative stress and dose-response characterization of pro-oxidant chemicals.

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LONG-TERM EFFECTS OF ^{137}Cs γ -RAYS ON INDUCTION OF CONGENITAL ANOMALIES IN RATS

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Complete information on the effects of radiation, the sensitivities to various doses of radiation, and the stage of gestation in pregnant female mammals at which radiation-induced congenital anomalies occur are important for our understanding of the teratogenesis or mutation process in mammals and for the evaluation of teratogenic and genetic hazards to humans.

The release of various radionuclides into the environments after the accident at the Chernobyl nuclear power station has caused the need to study the teratogenic effects of the most widespread radionuclides in mammals. The Chernobyl accident showed the consequences of releasing radioactive isotopes into the environment and the food chain. Radioisotopes with long half-lives, such as ^{137}Cs , are of greatest concern and present an enduring problem. To predict the teratogenic effects of ^{137}Cs on human beings, it is necessary to study the teratogenesis induced by this radionuclide in nonhuman mammals such as the rat. The purpose of the present study is to evaluate the teratogenesis caused by ^{137}Cs γ -radiation in rats under long-term exposures. Many congenital anomalies are caused by radiation or environmental factors, and it is likely that the assessment of teratogenesis in the present study will be very important in the future. Female Donryu strain rats were irradiated with ^{137}Cs γ -rays on days 9-18 of gestation. The animals were sacrificed on day 18 of gestation and the contents of each uterine horn were examined. The numbers of surviving, dead, and resorbed fetuses were recorded. The fetuses were examined for external and visceral malformations. The present experiment also measured the relative biological effectiveness (RBE) of tritiated water compared to ^{137}Cs γ -rays for the induction of developmental anomalies in pregnant female rats of the Donryu strain rats. The study was designed to estimate the incidence of developmental anomalies in surviving rat fetuses. Radiation exposures were approximately 0, 1, 2, 3, 4, 5, and 6 Gy for both tritiated water and ^{137}Cs γ -rays. The incidence of developmental anomalies in these fetuses increased from 4% in the control group to 98-100% in groups exposed to higher radiation doses. The results were fitted to various models relating 50% malformation incidence to radiation dose using the raw data to estimate risk. The calculated RBE values for tritiated water

(tritium β -rays) compared to ^{137}Cs γ -rays ranged from 1.8 to 2.4. A best estimation of the RBE for this experiment was about 1.9.



EFFECT OF CHRONIC LOW LEVEL EXPOSURE TO JET FUEL ON POSTURAL BALANCE OF U.S. AIR FORCE PERSONNEL

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The purpose of this pilot study was to identify potential relationships between increased postural sway and cumulative jet fuel exposure to U.S. Air Force (USAF) personnel. The specific aims of the study were: (a) to compare postural balance differences between an exposed population and a non-exposed population, and (b) to assess the relationship between cumulative exposure and changes in postural sway variables. The USAF bases selected for this study contained a population of workers potentially exposed to JP-8 jet fuel from depot level maintenance activity. The exposure group had a mean age of 37.5 years, ± 1.8 SEM and mean exposure of 12.0 years ± 1.4 SEM. An unexposed group from the bases and other locations with mean age 34.5, ± 1.7 SEM was used for comparison. Individual cumulative exposure was determined from personal air samples collected on charcoal tubes. All participants completed work and health history questionnaires to support calculation of exposure history and identify confounders. Mean exposure concentrations for daily time weighted average personal samples were noted for: total jet fuel (naphthas = 0.54 ppm ± 0.12 SEM), and jet fuel residual solvents (benzene [0.006 ± 0.001], toluene [0.01 ± 0.003] and m,o,p xylene [0.008 ± 0.002]). Mean cumulative exposure levels were: naphthas (1308 ppm ± 292 SEM), benzene (21.2 ± 5.7), toluene (23.8 ± 6.1), and m,o,p xylene (22.7 ± 5.4). A portable

microcomputer-based force platform system was used to collect postural sway data using the following series of tests for 30 seconds each: (a) EO - eyes open, standing on platform - which tested the visual, proprioceptive, and vestibular systems for controlling postural sway; (b) EC - eyes closed, on platform - which removed the visual system; (c) FO - eyes open, on 4 inch foam over platform - which modifies the proprioceptive system; and (d) FC - eyes closed, on 4 inch foam - which removed the visual system and modifies the proprioceptive system allowing the vestibular system to act as the primary control of postural sway. Results of a one-tail T-test for group comparison of mean log postural sway area indicated a positive, marginally significant ($p=0.059$), correlation for the EO test. The exposed workers had a 40% increased mean log postural sway area as compared with the non-exposed subjects. Covariate adjusted regression analysis of the exposed group identified a significant association ($p<0.05$) between residual solvents (benzene, toluene, and xylenes) and increased postural sway response. The most significant results were observed in JP-8 benzene model for sway length which had a P-value range of 0.0005 to 0.01 for all sway tests. The FC test, which implicates vestibular effect, was the most significant test for cumulative solvents exposure with a P-value range of 0.006 to 0.05 for sway length and 0.029 to 0.045 for sway area. No significant relationship was noted between naphthas and any of the postural sway variables. The findings of this evaluation support the conclusion that chronic, low-level exposure to solvents may produce subtle, long-term neurological effects as manifested by increased postural sway. Future in-depth study with greater power (increased sample size) is needed to better characterize and determine long-term health effect implications and intervention ideas.

Note: this is an expanded draft of the abstract being submitted to the AIHA for

presentation in the AIHC student poster session (May 96).



PROPOSED BIOLOGICAL MARKERS OF EXPOSURE, EFFECT, AND SUSCEPTIBILITY TO HYDRAZINES

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Hydrazines are used as propellants throughout the military and aerospace community, in multiple systems. H-70, a mixture of 70% hydrazine (N_2H_4) and 30% water, is used in the Emergency Power Unit (EPU) of approximately 1,600 F-16 aircraft worldwide. Unsymmetrical D-methyl Hydrazine (UDMH) is used in the fourth stage of the LGM118-A "Peacekeeper" missile. Hydrazines are also encountered in boiler systems and coolants of nuclear reactors aboard Naval vessels. Hydrazine exposures in a military setting are difficult to characterize, since the highest levels result from unplanned releases in an operational environment. For these reasons, the development of appropriate biological markers of exposure, effect, and susceptibility to hydrazines has been identified as a research priority by the Air Force Occupational Technology Needs assessment. (ESOH-TPIT, HSC/XRE needs F95-265, 987, and 2303). Other sources of potential hydrazine exposure include Environmental Tobacco Smoke, edible mushrooms, and a wide variety of azo-derived pharmaceuticals used as antidepressants, antineoplastics, antihypertensives, antihistamines, and tuberculostatics (INH). Because of requirements for worldwide duty, military personnel undergo periodic tuberculosis screening, and receive prophylactic isoniazid (INH) treatment for six months after showing positive skin tests. The presentation will discuss a proposed prospective cohort study of patients receiving isoniazid (INH), designed to evaluate several biological

markers of hydrazines. Markers of dose: plasma, urine, and expired hydrazines. Markers of effect: alpha, mu, pi-class glutathione-S-transferase, RBC calmodulin and calmodulin N-methyltransferase, ALT, AST, GGT, GGT-globulin, total protein, A/G ratio, HPRT mutant frequency (genotoxicity). Markers of susceptibility: acetylator genotype (NAT-2) and phenotype (caffeine clearance), vitamin B₆.

1. Agency for Toxic Substances Disease Registry (ATSDR), "Toxicological Profile for Hydrazines" (1994).



CHANGES IN INSULIN RECEPTOR BINDING AS A BIOMARKER

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A xenobiotic is generally defined as a compound not found normally in the body. If a xenobiotic has no selective target, a biomarker could exist that will reflect the integrated response of the organism. Also, the expression of early cell damage in one organ system should correlate well with changes in other systems. Therefore, for basic research studies, the biomarker chosen should be one which plays a role in all cells. Recently, hormonal effects has attracted interest for use as biomarkers in environmental toxicology. Insulin, the hormone responsible for glucose regulation and cell growth was chosen in this study. There were two aims: first, to measure insulin receptor binding using spin-labeled insulin; and, second, basic research on changes in the insulin receptor binding of human erythrocytes after exposure to radiation and chemicals likely to be encountered by military personnel. Insulin was spin-labeled and incubated with red blood cells exposed to radiation or chemicals, the cells were centrifuged and insulin receptor binding measured by double

integration of the spectra using a Bruker ESP300E spectrometer. Insulin receptor binding was decreased by radiation, nitrogen mustard, and exposure to ammonium dinitramide. The application of insulin receptor binding is likely to be useful for risk assessment efforts and the spin label technique could be developed as a clinical tool not just for insulin but for other compounds with specific protein receptors on cell membranes.



QUANTITATION OF FREE RADICALS IN B6C3F1 MOUSE LIVER SLICES ON EXPOSURE TO FOUR CHEMICAL CARCINOGENS: AN EPR/SPIN TRAPPING STUDY

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Free radicals generated in mouse liver slices following exposure to four chemical carcinogens: tert-butyl hydroxide (TBOOH), bromotrichloromethane (BrCCl₃), carbon tetrachloride (CCl₄), and trichloroethylene (TCE), were quantitated using electron paramagnetic resonance spectroscopy (EPR)/spin trapping techniques. Precision cut liver slices (n=128) were prepared from B6C3F1 mice and incubated in Waymouth's media supplemented with 10% fetal bovine serum and 10 mM N-tert-butyl- α -phenyl nitron (PBN). Liver slices were exposed to control media or media with 1 mM TBOOH, BrCCl₃, CCl₄, or TCE. After 5 or 60 min. the slices and media were homogenized, frozen in liquid nitrogen and lyophilized. Radicals trapped by PBN were quantitated using an EMS 104 electron paramagnetic analyzer (Bruker Instruments, MA). Standards were prepared by homogenizing liver slices with known quantities of the spin label 2,2,5,5-tetramethyl-1-pyrrolidinyloxy-3-carboxamide (3-CAR). EPR spectra were compared quantitatively and qualitatively. The EPR spectra of the lyophilized liver homogenate

suggest there is a difference in the radical species trapped if slices are incubated in media containing the four carcinogens when compared to control media. The interaction of both time and concentration should be addressed in any studies designed to quantitate free radical-induced liver damage.



ORAL BIOAVAILABILITY OF TPH AND OTHER CHEMICALS IN SOIL: LITERATURE REVIEW OF EXPERIMENTAL ISSUES AND RISK ASSESSMENT APPLICATIONS

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Total Petroleum Hydrocarbon (TPH) contamination of soil is a problem at Air Force bases nationwide, causing it to be a major environmental clean-up concern. Human exposure to TPH in the soil can occur through several pathways, including ingestion of soil or sediment particles. Exposure through ingestion of TPH contaminated soils can be investigated using oral soil dosing studies. This poster focuses on the methods used in soil dosing studies, the effects of soil on the bioavailability or toxicity of contaminants, and the potential use of bioavailability information in risk assessments and the development of risk-based clean-up of sites.



CONTINUOUS EXPOSURE MONITORING TO IMPROVE HUMAN HEALTH RISK ASSESSMENT

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Of the 1416 sites currently on the Environmental Protection Agency's National Priorities List, more than 100 of these hazardous waste sites belong to the Department of Defense. These sites plus other sites where hazardous wastes or

chemicals are handled often require a human health risk assessment (HHRA) to help determine clean up or management strategies which are protective of human health. The Department of Defense (DOD) and other government agencies have long recognized the need for a more realistic assessment of exposure risk than is presently available via traditional HHRA procedures. This poster will demonstrate how human health data, real-time monitoring, and continuous monitoring methods can be used to obtain more precise measures of human exposures, and thus provide a better understanding of the public health risk to communities located near to and employees at a hazardous waste site. This more definitive risk assessment includes a public health perspective, expanded toxicity and exposure assessments, and a more robust risk characterization based upon more conclusive data.

Open-path FT-IR (Fourier Transform Infrared) spectroscopy can be used to provide real-time continuous monitoring (as opposed to infrequent periodic 24-hr sampling techniques) of effluent streams emanating from facilities that deal with hazardous wastes. Battelle successfully developed an open-path FT-IR at Tinker Air Force Base to monitor airborne Volatile Organic Compound (VOC) emissions from the industrial wastewater treatment plant. In this instance, FT-IR provides constant monitoring protection at the treatment plant fenceline. Exposure levels determined by FT-IR measurements can be extrapolated to a lifetime risk factor and be compared with established risk values.

The information provided by different innovative monitoring techniques like FT-IR and other real-time technologies for VOC measurements strengthens the exposure assessment. These data plus relevant health data, and in-depth toxicity evaluation, provide a human health risk assessment based on more definitive data which results in better

decisions about the site. Ultimately, this approach can beneficially affect the resolution of community health concerns, clean-up and management strategies, and the cost of hazardous waste management and remediation for the Department of Defense.



A STATISTICAL METHOD FOR DETECTING DEPARTURES FROM ADDITIVITY FOR USE IN ESTIMATING TOXIC INTERACTION EFFECTS ASSOCIATED WITH EXPOSURES TO TOLUENE AND BENZENE MIXTURES

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The U.S. EPA has pursued the estimation of risk of adverse effects from exposure to chemical mixtures since the early 1980s. Initial methods to calculate risk estimates were often component based approaches that required assumptions of dose-addition or response-addition and ignored possible changes in response due to interaction effects. In this research, a dose-response model is built for the mixture of concern under the assumption of additivity, using single chemical dose-response information on the individual components of the mixture. For any specific combination of these components, then, the risk of an adverse effect from exposure to the mixture can be estimated, assuming no interaction effect. Then, a laboratory experiment can be conducted on that specific composition of the mixture of concern, and a comparison can be made between this predicted risk estimate and the laboratory results. From this, it may be determined whether the dose-response of the laboratory data is greater than or less than additive, i.e., the model's prediction, and

by what magnitude. In conjunction with the development of this statistical theory, experimental data have been generated for use in testing this model. These data include time to developmental stage, heart rate progression, and lethality in Medaka fish embryos exposed to benzene and toluene individually and as binary mixtures. Results include an analysis of the combined toxic effect (lethality) of these chemicals, of adverse effects on developmental stage and heart rate (heartbeat) progression, and of where departures from additivity have occurred.



REGRESSION EQUATIONS TO ESTIMATE PROVISIONAL TLV/WEEL EQUIVALENT FOR NON-CARCINOGEN CHEMICALS HAVING NO TLV OR WEEL

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The number of chemicals released by human activities to the natural environment and the built environment (including most workplaces) far outweighs the number of chemicals in commerce which have been toxicologically evaluated to develop a professional society guideline - against which to measure hazard to worker health. Guidelines most often used in North America are TLVs (ACGIH) and WEELs (AIHA). The goal was to develop a risk-based exposure guideline process, where there is need, and a TLV or WEEL has not been assigned. The combined TLV-WEEL list is about 700 chemicals. The informational database IRIS and the HEAST tables, both from the U.S. EPA, provide Agency-sifted information on risk in the form of thresholds (NOELs, LOAELs), for both oral and inhalation routes, for non-carcinogens. For a couple of hundred chemicals, at least some IRIS/HEAST and TLV/WEEL information

existed. Several regression equations have been run, to provide a way to estimate a TLV/WEEL-equivalent value, where none exists, based on those chemicals already evaluated in both systems. Findings: (1) inhalation NOELs and LOAELs correlate better with TLV/WEELs than do oral toxic thresholds, and (2) primary threshold measurements correlate better with TLV/WEELs than do oral RfDs and inhalation RfCs. Poorer correlation with Reference doses (concentrations) suggests either that the U.S. EPA might re-examine its assignment of safety factor values, or that the ACGIH/AIHA might re-examine the relation of their exposure criteria to literature threshold measurements. Assumptions, e.g., regarding the diversity of toxic endpoints, and limitations of the method will be reviewed, as well as potential uses. The relevance to environmental health is indirect but important. The best strategy to protect environmental health is pollution prevention, an important part of which is safer material substitution in industrial processes. In order to be duly diligent, substitution must quantitatively evaluate hazard to both the environment and workers, as well as cost, effectiveness of product, and other input to management decisions on substitution. This work contributes to that effort as an integral part of a newly developed worker hazard score, to be used together with an environmental hazard score, such score separate for each chemical to be replaced and for each candidate substitute, to provide quantitative hazard input to management decisions on how to reduce generation of pollution and wastes.

Work supported by Indiana Pollution Prevention and Safe Materials Institute, Purdue University.

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USE OF PROBABILISTIC RISK ASSESSMENT AT U.S. AIR FORCE INSTALLATIONS

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Risk assessment approaches currently used by U.S. Air Force (USAF) Installations within the United States and accepted by the U.S. EPA are based on deterministic (single-value) risk estimates. Probabilistic risk assessment is coming to the forefront because it allows the risk assessor input ranges of exposure values characterizes the uncertainty of the input values. This poster presents a comparison of probabilistic and deterministic health risk estimates based on data from several USAF Installations in the United States. We considered exposure to volatile organic compounds (VOCs) from on-base industrial operations and the impact on community drinking water ingestion, dermal contact, and showering in the risk assessment. Probability densities functions used included concentrations, contact rates, and exposure frequencies; dose-response inputs will be single values. Deterministic risk estimates were calculated by the "reasonable maximum exposure" (RME) approach recommended by the EPA Superfund program. The comparison quantifies the protectiveness of EPA's RME estimates and suggests the potential usefulness of Monte Carlo simulation in implementing EPA's multiple risk estimates.

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DOSE (AND TIME DEPENDENT) BLOCKADE OF PREGNANCY IN SPRAGUE-DAWLEY RATS ADMINISTERED AMMONIUM DINITRAMIDE IN DRINKING WATER

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Ammonium dinitramide (ADN) is a class 1.1 explosive oxidizer, which may be used in solid rocket propellant mixtures and explosives. A 90-day general

toxicity/reproductive screen performed on this compound at doses of 162, 103, 29, and 0.0 mg ADN/kg/day resulted in a treatment-related adverse effect on litter production. Incidences of animals producing litters (1/11, 3/12, 12/12, and 11/12, respectively) and mean numbers of pups per litter (7, 7, 16, and 15, respectively) were both statistically significantly less than controls. In a follow-up study, mated dams treated with ADN at the same doses and examined at Gestation Days 10 and 20 showed a similar effect in fetus loss as those in the reproductive screen. A pre- versus postimplantation dosing regimen indicated that implantation is vulnerable to ADN effects during the preimplantation period (Gestation Days 1-3). No implantation sites were found in the rats treated with 2000 mg ADN/L drinking water during Gestation Days 1-3. Numbers of implantation sites found in the rats treated during Gestation Days 4-8 were similar to those found in the control group. The pituitary was not specifically identified in this study as the site of primary action, but serum progesterone was reduced by 27%, which may have resulted in reduced fertility.

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REPRODUCTIVE SCREEN OF MODULAR ARTILLERY CHARGE SYSTEM ADMINISTERED IN THE DIET OF SPRAGUE-DAWLEY RATS

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An artillery propellant under development by the U.S. Army is a granular mixture of 98% nitrocellulose, nitroglycerin, nitroguanidine, and <2% cryolite and graphite. The propellant, Modular Artillery Charge System, or MACS, consists of a single increment of propellant charge contained within a rigid combustible casing. As part of the process to develop environmental and health effects criteria, a 90-day modified Screening Information Data Set reproductive assay was performed. Male and female Sprague-

Dawley rats were treated with diet containing either 0.0, 0.2, 1.0, or 2.0 g propellant/kg diet. No mortalities occurred and body weights were unaffected by treatment. Methemoglobin concentrations of the high-dose rats measured at 28 days and at the conclusion of the study were significantly elevated (23 to 25%) compared to control rats. Relative organ weights of treated animals did not differ from weights of their respective control groups. No adverse effects occurred in mating or fertility indices. No significant treatment-related differences were noted in length of gestation, sex ratio, gestation index, or mean number of pups per litter.

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DEVELOPMENT OF THE RESRAD FAMILY OF CODES FOR ENVIRONMENTAL RISK ASSESSMENT

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Argonne National Laboratory, under the sponsorship of the U.S. Department of Energy (DOE), has developed a series of computer codes for environmental risk assessment. These computer codes can be applied to evaluate sites contaminated with radioactive materials and hazardous chemicals. The first in the series is the RESRAD code, which was developed in the 1980s and was officially released for public use in June 1989. RESRAD is a pathway analysis computer code designed to calculate radiation doses and cancer risks to a critical population group and to derive cleanup criteria for radioactively contaminated soils. It was designated by DOE in Order 5400.5 for use in evaluating compliance with DOE residual radioactive material guidelines. Since its release, RESRAD has been widely used by DOE, other agencies, and their contractors.

The second in the series is the RESRAD-CHEM code, a sister code of RESRAD that was designed for chemical risk assessment. The RESRAD-CHEM code implements the same methodology as the RESRAD code except that it also considers inhalation of volatile compounds and dermal absorption pathways, but excludes the external radiation pathway. Currently, there are 151 chemicals in the RESRAD-CHEM database.

RESRAD-BUILD was designed specifically to evaluate buildings contaminated with radioactive materials. A variety of building compartments and contamination forms are modeled with corresponding exposure pathways. Examples are the transport of contaminants from one room to another via air ventilation and the penetration of gamma radiation through walls and ceilings.

To ease calculation efforts, the RESRAD-BASELINE code was developed to perform baseline risk assessments following the U.S. Environmental Protection Agency's human health risk assessment guidelines. This computer code allows the user to input environmental media concentrations and calculates the corresponding radiation doses and cancer risks. The user can simulate different exposure scenarios by selecting relevant pathways and entering appropriate consumption and exposure parameters. Another in the series is the RESRAD-RECYCLE computer code, which estimates radiation doses to various receptors resulting from the recycle and/or reuse of radioactively contaminated materials/equipment.

In addition to the evaluation of human health risk, RESRAD-ECORISK estimates the risk from contaminant exposure to ecological receptors. This code is based on the methodology of RESRAD-CHEM and uses the species-specific life history information to calculate an ecological effects quotient and estimates risk to ecological receptors of concern. Currently five ecological receptors

including two bird and three mammal species are considered in RESRAD-ECORISK.

The trend in environmental risk assessment is to estimate the uncertainties associated with predicted risks. A new version of the RESRAD code, RESRAD-Probabilistic, was developed to quantify these uncertainties. This code allows the user to input parameter distributions. The output of RESRAD-Probabilistic includes graphic illustration of cumulative probabilistic dose distribution and tabular listings of statistical results.

All of the RESRAD family codes have user-friendly interfaces and provide on-line help messages through their operation. They are flexible for different applications, are maintained and updated regularly, and are very easy to use. Several documents have been prepared to support their operation.

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PHARMACOKINETICS AND PHARMACODYNAMICS OF 3,3',4,4'- TETRACHLOROBIPHENYL IN THE RATS: PBPK MODEL DEVELOPMENT

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Lipophilic and stable polychlorinated biphenyls are ubiquitous environmental contaminants and exposure of PCB77 to the rats induces CYP1A1 enzyme activity. The objectives of this study were (1) to investigate the disposition and hepatic enzyme induction in the rats following a single ip injection of PCB77 and (2) to develop and validate a PBPK model. Male Fischer 344 rats (n=4) were dosed with radiolabeled PCB77 (0.5 mg and 5 mg/kg bw) in corn oil and control rats received corn oil only. At low doses, the exposed rats were sacrificed at eleven time points till Day 16 and at high doses twelve time points till Day 44. Slow uptake and clearance in the fat were described by a diffusion-limited process while other tissues were described by a flow-

limited process. The ratio of PCB77 concentrations in the liver to fat was time-dependent and PCB77 concentration was higher in the fat than liver after Day 1 post dosing. Uptake of PCB77 to the systemic system and elimination of PCB77-derived radioactivity in the feces were described as the first order process. Induction of hepatic ethoxyresorufin O-deethylase (EROD), a biomarker for CYP1A1, was dose-dependent and Michaelis-Menten-type equation was used to describe EROD induction. Rats were exposed to six different concentrations of PCB77 (0.1, 0.5, 1, 5, 10, and 50 mg/kg bw) and sacrificed them on Day 3 to validate the PCB77 model. Model estimation of kinetic behavior in the liver and fat, and EROD induction were adequately described by the model except for the 50mg/kg bw.

ABSTRACTS FOR DATABASES

DATABASE SESSION ABSTRACTS
CONFERENCE ON ADVANCES IN TOXICOLOGY AND
APPLICATIONS TO RISK ASSESSMENT

(PRESENTER UNDERLINED)

**RESRAD-ECORISK: A COMPUTER CODE FOR CONDUCTING RAPID SCREENING LEVEL
ECOLOGICAL RISK ASSESSMENTS**

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**HAZARDOUS MATERIAL PERSONAL COMPUTER LOCAL AREA NETWORK (HAZMAT PC-
LAN)**

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INTERNATIONAL TOXICITY ESTIMATES FOR RISK (ITER)

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Toxicology Excellence for Risk Assessment (TERA), Cincinnati, OH

ENVIRONMENTAL HEALTH AND SAFETY SERIES

Maynard Short

Micromedex, Inc., Englewood, CO

RESRAD-ECORISK: A COMPUTER CODE FOR CONDUCTING RAPID SCREENING LEVEL ECOLOGICAL RISK ASSESSMENTS

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RESRAD-ECORISK is a computer model for estimating current and future ecological risks from chemically contaminated sites. Beginning with a contaminated soil source, the model predicts environmental concentrations in other media using site-specific characterization data and fate and transport models. The code then follows a pathway-analysis approach and employs species-specific exposure models to estimate daily uptake of chemicals by ecological receptors along multiple exposure pathways. Results calculated by RESRAD-ECORISK include 1) environmental media concentrations over time, 2) applied daily doses for each contaminant, pathway, and receptor, 3) ecological hazard quotients for each contaminant and receptor, and 4) preliminary remediation soil guidelines for each contaminant and receptor. The code currently includes five ecological receptors: American robin, deer mouse, mallard duck, white-tailed deer, and eastern cottontail. Chemical and ecological databases are included to provide default values for input parameters. The code is easy to install and runs on a 486 or higher PC. It includes a user-friendly interface with on-line help files, can evaluate multiple chemical and receptors in a single run, and will generate a text report presenting the results. The user may select or suppress exposure pathways as appropriate, and may modify ecological exposure factors as needed.



HAZARDOUS MATERIAL PERSONAL COMPUTER LOCAL AREA NETWORK (HAZMAT PC-LAN)

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The HAZMAT PC-LAN is comprised of a NOVELL based network utilizing commercial-off-the-shelf software applications and standard personal computer equipment. It is a Windows based system that is accessed using pcAnywhere Remote Version 2.0 for Windows, for which we have a site license for all AFMC installations. The HAZMAT PC-LAN provides workplace access to hazardous material information via a menu driven system which utilizes local area networks and local dial-in modems. It utilizes the AFMCe Virtual Circuit Switch which allows access to a centralized file server housed at Wright-Patterson AFB. The VCS Network utilizes AFNET trunks to pass long-haul data between Wright-Patterson AFB and the ALCs.

Users access the HAZMAT databases locally via their local base wideband LANs and local dial-up connections. The local dial-up connections eliminate long distance charges previously associated with centralized database operations.

The HAZMAT PC-LAN provides access to the following databases:

Image Database Management System.
This is a Windows based application that manages the entry, storage, and retrieval of Material Safety Data Sheet data in the form of scanned images.

The Defense Logistics Agency's Hazardous Material Control and Management/Hazardous Materials Information System is a hazardous material information CD-ROM sponsored by the Defense General Supply Center. The HMIS database provides access to NAVY Defense Logistics Agency's Proprietary and Non-Proprietary Material Safety Data Sheets which provide information pertinent to exposure, treatment, and care in the handling of hazardous materials. Searches are

performed by FSC, National Item Identification Number, Manufacturer's CAGE Number, Part Number Indicator, and Part Number/Trade Name.

Information Handling Service's PartsMaster database contains DOD procurement information. PartsMaster allows access to procurement record data by performing database searches by National Stock Number, National Item Identification Number, Manufacturer's Part Number, Company Number, AMDF Line Item Number, SPCC Navy Item Control Number, Navy Manufacturer's Part Number, MIAPL Identification Number, MIAPL Repair Item Code, and item name singly or in combination.

Micromedex's Toxicological, Occupational Medicine and Environmental Series Database, TOMES Plus, is an industrial chemical database that provides access to medical and hazard information which is needed for safe management of chemicals in the workplace, evaluating exposures, right-to-know issues for SARA Title III regulatory situations, and environmental incidents.

The Occupational Safety and Health Administration and the Environmental Protection Agency Code of Federal Regulations.

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INTERNATIONAL TOXICITY ESTIMATES FOR RISK (ITER)

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International Toxicity Estimates for Risk (ITER) is a compilation of human health risk values for chemicals of environmental concern from health organizations worldwide. ITER is a project of Toxicology Excellence for Risk Assessment (TERA), a non-profit risk assessment firm dedicated to

the best use of toxicity data in developing risk assessment values. TERA staff compile risk values from existing publications of health organizations into a consistent format, so that comparisons can readily be made by informed users. Details are provided on the basis for cancer classifications and quantitative risk values and users are directed to each organization's source document/information system for further details. TERA compares the values and explains the source of differences.

A pilot of ITER is currently available on the World Wide Web (<http://www.tera.org>) and includes approximately 20 chemicals with over 100 toxicity values and cancer classifications from three organizations: Health Canada, U.S. EPA, and the U.S. ATSDR. Additional chemicals and organization's values will be added as funding is secured.

ITER will also include independently-derived values which have undergone external peer review at a TERA-sponsored peer-review meeting. These meetings will include a panel of experts chosen to balance any potential biases due to employment or other issues. TERA prevents conflicts of interest in part through its nonprofit status and policy of informed and neutral guidance.

The information on ITER is useful to risk assessors and risk managers needing human health toxicity values to make risk-based decisions. Other sources of these data generally contain values from just one organization and sometimes contain outdated values or values that do not make full use of all toxicity data. ITER allows the user to compare values from different organizations to determine the best value to use for the human exposure situation being evaluated.

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ENVIRONMENTAL HEALTH AND SAFETY SERIES - MICROMEDEX, INC.

Maynard Short
Micromedex, Inc., Englewood, CO

Environmental Health and Safety Series is a comprehensive chemical reference providing medical and hazard data on thousands of substances. Its proprietary and government databases assist professionals concerned with protecting the health and safety of people, the workplace, and the environment. CD-ROMs for PCs and LANs, and tapes for mainframe computers, are available.

Chemmate from MICROMEDEX - Windows™ based software product analyzes chemical products and mixtures, then writes Material Safety Data Sheets (MSDS), Health and Safety Data Sheets, warning labels, and signs in compliance with national/international safety regulations. It automatically creates customizable English, French, and Spanish documents in a variety of international formats.