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Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX) is a 96-hour, whole-embryo bioassay designed to detect potential developmental toxicants. The primary goal of this research was to improve the predictability and increase the overall utility of FETAX as a screen for developmental toxicants that pose a hazard to human health. The first specific aim was to explore the use of phenobarbital, β -naphthoflavone, and isoniazid-induced rat liver microsomes as an exogenous metabolic activation system. This particular inducing system supplanted the presently-used Aroclor 1254 system which was recently proven to be somewhat unreliable. Post-isolation mixtures of these microsomes represented a broader spectrum of P-450 isozymes, and thus, bioactivated/inactivated a wider range of compounds. The second specific object was to develop and evaluate a standardized metabolic activation system test kit for FETAX which could be used by other FETAX users, including the U.S. Army. The findings from this research demonstrated that the post-isolation mixed microsomes were highly effective and that the test kits provided a reliable standardized means of routinely using the exogenous activation system with FETAX.

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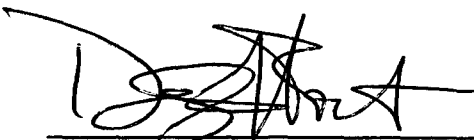
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Introduction

The vast majority of the epidemiological evidence acquired today suggests that most birth defects are the result of non-genetic environmental factors. Approximately 100,000-150,000 chemicals are currently available in the market place with 2,500 new chemicals released each year. Prior to their release into the market place and ultimately the environment, the safety of these compounds must be firmly established. The expense and time-consuming nature of conventional testing has warranted the development, validation, and eventual wide-spread use of alternative, non-mammalian model systems. FETAX (Frog Embryo Teratogenesis Assay - *Xenopus*) is a 4-day, non-mammalian, whole-embryo bioassay designed to evaluate potential teratogenic hazard by: 1) direct chemical/complex environmental mixture screening, and 2) use as a tool to evaluate toxicological mechanisms of action. FETAX has been extensively validated as a screening assay using both pure chemical compounds and complex environmental mixtures (wastewater effluent, sediments, and groundwater). Because *Xenopus* lack many metabolic enzyme systems including the mixed-function oxidase (MFO) system through the first 96-hours of development, an exogenous metabolic activation system (MAS) was developed (1) and evaluated (2-7) using Aroclor 1254-induced rat liver microsomes. However, the use of alternative, more advantageous inducing agents with the rat liver microsomes have only recently been explored with the FETAX assay.

Of approximately three million infants born alive each year, 13.3 per 1000 die within the first year (8). Approximately 2 to 3 percent of infants born alive have major congenital malformations recognized within the first year of life (9). When defects that only become apparent later in life are included, the frequency of major and minor malformations increases to about 16 percent (10). Approximately 7 percent of newborns are born prematurely (before the 37th week) and 7 percent of infants born at full term have low birth weights (2.5 kg or less) (11).

The NIOSH Registry of Toxic Substances (12) indicated that of some 38,000 chemicals in common industrial use, only 1,930 of these had been tested for teratogenicity with 580 demonstrating positive effects. In the Catalog of Teratogenic Agents compiled by Thomas Shepard (13), over 600 chemical agents are presented with only 20 known to induce birth defects in humans. Obviously many gaps exist in our knowledge of how to predict which chemicals, among the million present in our environment or workplace, possess teratogenic potential; and the correlation between results in animal studies and effects in humans.

Developing an exogenous MAS for FETAX which is not dependent on Aroclor 1254 induction is imperative if FETAX is to be widely effective and universally accepted as a surrogate developmental toxicity screening assay. The use of Aroclor 1254 as an inducing agent presents several problems including: 1) inconsistent induction due to congener concentration variability from lot to lot, 2) isozyme repression (CYP2E1), 3) not readily available due to reduction in the use of PCBs, and 4) hazardous waste and human health hazard issues. Thus, developing post-isolation mixtures of rat liver microsomes induced by β -naphthoflavone (CYP1A1 and 1A2), phenobarbital (CYP2A6/2B6), and isoniazid (CYP2E1) will help alleviate the problems associated with the use of Aroclor 1254 while providing a broad-spectrum of induced isozymes. The

previous mentioned isozymes represent the primary P-450 isozymes responsible for Phase I biotransformation of potential developmental toxicants. Additional inducing agents may be added to this repertoire as needed in the future.

By evaluating FETAX as a developmental toxicity screening system, the U.S. Army, scientific community, chemical and pharmaceutical producing industries, appropriate regulatory agencies, and ultimately the public will be provided with a versatile alternative, short-term prescreening assay and mechanistic model for the commonly used three segment test protocol. As opposed to many other advance technologies this bioassay can be developed, validated, and utilized quickly.

The *Xenopus* test system is a non-mammalian, alternative assay which offers some short and long-term solutions to current problems associated with developmental hazard evaluation. The problems are: increasing regulatory requirements, test cost, test duration, and the reduction in the use of mammals in research. Existing *in vivo* mammalian test systems are effective for the testing of drugs and cosmetics, but are too lengthy, expensive and technically difficult for evaluating mechanisms of action. In addition, the number of chemicals in production which require testing is increasing at a rate faster than is feasible to handle with *in vivo* test systems. These events have produced a climate which favors the use of alternative test systems for eventual refinement and possible reduction of *in vivo* assays. In a study conducted for NIH, the National Academy of Sciences recently concluded that the development and use of lower life form models for toxicity of testing should be emphasized. Tighter restrictions will limit the use of *in vivo* assays and increase the costs associated with their performance. The Office of Technology assessment for the United States Congress has recently completed an assessment of the use of animals in testing, research and education. Congressional options emanating from this study include support for the further development and use of *in vitro* test systems when appropriate. Thus, cost-effective, predictive developmentally relevant, tests are urgently needed to allow the rapid evaluation of developmental toxicants in the environment and to elucidate toxicological mechanisms of teratogenesis.

The ultimate goal of the proposed research is to protect humans from developmentally toxic chemicals, regardless of where exposure may take place, by either direct aversion or by increasing the understanding of toxicological mechanisms of teratogenesis. Knowledge of toxicological mechanisms of actions of commonly used drugs by physicians and pharmacists should increase confidence in the administrations of these drugs during pregnancy. In addition, this information will allow chemists and biologists to make educated, well-informed decisions on the development and manufacture of chemicals by understanding potential toxicological effects.

The specific goal of the proposed research was to improve the performance of FETAX by developing a more efficacious MAS using mixtures of β -naphthoflavone-, phenobarbital, and isoniazid-induced rat live microsomes. Recent discussions with chemical and pharmaceutical manufacturing industries, as well as, the National Institute of Environmental Health Sciences (NIEHS), have indicated that these groups are interested in the FETAX system, but would not use or recommend the system on a large-scale basis unless the model could both screen for human developmental toxicants and elucidate mechanisms of action by including a standardized testing protocol with an effective MAS. Furthermore, the groups indicated that the screening assay must

be well developed and properly validated. Therefore, the development and proper validation of an exogenous MAS for FETAX must be performed before this assay can be successfully utilized.

The original work on developing a developmental toxicity screening assay using *Xenopus* embryos to detect environmental teratogens was performed in the laboratories of Greenhouse (14) and Dumont (15). Greenhouse used 48-hour exposures to military compounds (N-phenyl- α -naphthylamine and various hydrazines) to demonstrate toxic and teratogenic effects on developing embryos. Early studies in our laboratory have demonstrated that FETAX can be used with a variety of chemicals and complex mixtures. The endpoints included: LC50 (mortality), EC50 (malformation-teratogenesis), no observed effect concentration (NOEC), minimum concentration to inhibit growth (MCIG) [both length and developmental stage], motor behavior, pigmentation, and gross anatomy. A Teratogenic Index (TI), the ratio of the 96-h LC50/96-h EC50 (malformation) was developed and has been successfully used as a measure of the relative developmental hazard of a substance (1-7, 16-20). Thus, assessment of teratogenic potential has been based on TI values, embryo growth, and types and severity of induced malformations. Generally, TI values <1.5 have indicated low teratogenic potential, whereas higher values have signified an increase in the potential teratogenic hazard, as there was little or no separation between the concentrations that cause embryo malformation, but cause no embryo-lethal effects, and concentrations which were embryo-lethal. Greater TI values signified a larger separation between the two responses, and thus, a greater possibility of embryos being malformed in the absence of significant embryo lethality. Types and severity of induced malformations have also been considered, especially for compounds with TI values <1.5 which produce serious defects of major organ systems. Such compounds may still pose a serious threat, possibly as embryo-lethal agents. We are currently establishing a data base to assess the utility of the TI value in assessing teratogenic hazard. Test chemical exposures have been continuous for 96-hours. Mortality and stage of development were checked at hours 24, 48, 72, and 96, while the other endpoints were recorded only at hour 96. Test compounds renewal was performed daily throughout the tests. Data collection was simple, as all observations were made with a dissection microscope. The data collected using FETAX have been in harmony with the criteria for an *in vitro* teratogenesis screen suggested by Kimmel et al. (21). These criteria included: good concentration-response relationships, adequate number of embryos, and easily defined and quantified endpoints.

In addition, an American Society of Testing and Materials (ASTM) New Standard Guide for the Conduct of FETAX has been recently published (22). An "Atlas of Abnormalities" companion document to the ASTM guide has been produced in order to aid in the proper scoring of malformations (23).

FETAX Test Performance

Dumont (unpublished) has accumulated validation data on 45 compounds with nearly 85 percent correspondence to mammalian results. Sabourin (24) have completed testing of 32 compounds with 83% predictive accuracy. However, neither of these two investigators used the exogenous MAS developed for FETAX. With over 100 compounds tested, we have approached 95% predictive accuracy in our laboratory using the Aroclor 1254-induced exogenous MAS.

Correlation between laboratories has been encouraging, as well. For example, Courchesne and Bantle (25) found a teratogenic index for hydroxyurea of 4.3, whereas Sabourin recorded 4.5 for the same chemical. We have performed an extensive interlaboratory validation study with several laboratories across the United States (26-29). Results obtained were extremely encouraging and warranted further development and validation of a versatile, yet more robust MAS for FETAX.

Sabourin and Carlton (unpublished) determined that the same stock of diphenylhydantoin caused pericardial edema as the primary anomaly in both the cultured whole rat embryo and *Xenopus* embryos. Dumont et al. (30) similarly found that meclizine induced hydrocephalia in both frogs and mammals and that other teratogens produced similar abnormalities in both frogs and mammals (developmental mimicry). Courchesne and Bantle (25) reported that a number of genotoxic chemicals caused the same general types of malformations in both *Xenopus* and rodent embryos. Dawson et al. (16) have developed an artificial medium (FETAX solution) and have carried out a preliminary validation using five compounds ranging from a nonteratogen to a strong teratogen producing results corresponding to current mammalian literature. Fort et al. (1,3-7) have found that twenty-six known mammalian teratogens tested with FETAX produced similar malformations as observed in mammals.

Evaluation of Exogenous Metabolic Activation System

As mentioned in previous sections, the inability of young *Xenopus* embryos to metabolize xenobiotics prompted the development of an exogenous MAS. We developed the MAS by using Aroclor 1254-induced rat liver microsomes and an NADPH generating system co-cultured with the embryos. The extensively studied proteratogenic compound, cyclophosphamide (31), was used in the initial pilot study to assess the feasibility of the system (1). Results indicated that cyclophosphamide was bioactivated into an embryotoxic form using the exogenous MAS. Also, addition of the microsomes, antibiotics (used to combat bacterial contamination) and a NADPH generating system did not increase mortality and malformation rates above FETAX solution control values. Specifically, activation reduced the 96-hour LC50 of cyclophosphamide 5.7-fold from 8.0 to 1.4 mg/mL. The 96-hour EC50 (malformation) was reduced 15.5-fold from 6.2 to 0.4 mg/mL. The 96-hour TI was increased 2.7-fold upon activation from 1.3 to 3.5. The severity of the malformations were used in FETAX compared to uninduced rat liver microsomes and generating system (32).

Additional validation studies were performed with Aroclor 1254-induced microsomes co-cultured with *Xenopus* embryos (3). Rifampicin, 2-acetylaminofluorene, and benzo(α)pyrene were all bioactivated as expected, while cytochalasin D was inactivated. The inclusion of the MAS had no effect on the developmental toxicity of zinc as expected. Nicotine had a TI value of 325 when tested in FETAX without Aroclor 1254-induced rat liver microsomes (2). This value was reduced to 3.5 when the embryos were co-cultured with an Aroclor 1254-induced MAS. However, bioactivation did not always result in a change in the TI value. Bantle et al. (33) found that metabolic activation reduced the 96-hour LC50 and EC50 (malformation) of N-ethyl-N-nitrosourea by a factor of 73, but did not change the TI value.

Evaluating Toxicological Mechanisms of Action

Preliminary studies have demonstrated the utility and versatility of FETAX for evaluating the toxicological mechanisms of action of diphenylhydantoin (4). In the initial study, teratogenic potentials of diphenylhydantoin (DPH) and hydroxylated metabolites (HPPH) were evaluated with FETAX. *Xenopus* embryos were exposed to DPH and HPPH in two separate static-renewal experiments with and without the presence of an exogenous MAS for 96 hours. Two separate concentration-response tests were also conducted with DPH and HPPH with a MAS modulated by various MFO inhibitors [carbon monoxide (CO) (broad-spectrum cytochrome P-450), cimetidine (mainly cytochrome P-450), ellipticine (cytochrome P-4490), and an epoxide hydrolase inhibitor (cyclohexene oxide)]. Assessment of the potential teratogenic hazard was based on TI values, types and severity of malformations, and embryos growth endpoints. Addition of the intact MAS to DPH increased the 96-hour LC50 and EC50 (malformation) from approximately 74.5 and 32.4 mg/L to 126.4 and 62.4 mg/L, respectively. The TI was reduced 1.2-fold. Both *p*-HPPH and *m*-HPPH were much less developmentally toxic than DPH. CO and cimetidine inhibition of cytochrome P-450 maintained much of the developmentally toxicity of DPH, whereas ellipticine inhibition of cytochrome P-448 was much less effective in maintaining the developmental toxicity of DPH. Cyclohexene oxide inhibition of epoxide hydrolase markedly increased DPH-induced embryotoxicity decreasing the 96-hour LC50 from approximately 74.5 to 38.6 mg/L. These results suggested that unmetabolized DPH and an embryotoxic epoxide intermediate served as the teratogenic species in FETAX.

In a separate study, the developmental toxicity of isoniazid (INH) and the metabolites acetylhydrazide (AH) and isonicotinic acid (INA) were examined with FETAX (5). Late *Xenopus* blastulae were exposed to INH, AH, and INA for 96-hours in two separate static-renewal tests with and without the presence of three differently induced metabolic activation systems (MAS). The MAS consisted of uninduced, Aroclor 1254-induced, and INH-induced rat liver microsomes. Addition of the INH-induced MAS decreased the 96-hour LC50 of INH and AH approximately 1.6-fold and 7.0-fold, respectively. The 96-hour EC50 (malformation) of INH was virtually unaffected; however, the INH-MAS decreased the TI value nearly 1.8-fold. The 96-hour EC50 (malformation) of AH increased approximately 2.0-fold, decreasing the TI value 15.9-fold. INA yielded a TI value of 2.5. Neither the uninduced MAS nor the Aroclor 1254-induced MAS had an affect on any of the compounds tested and none of the MASs affected the developmental toxicity of INA. Results from this study suggested that MFO metabolism may have altered the developmental toxicity of INH *in vitro* by producing a more embryo-lethal, but less teratogenic metabolite(s) than INH or AH themselves.

Finally, potential mechanisms of acetaminophen-induced developmental toxicity were evaluated using FETAX (7). Early *Xenopus* embryos were exposed to acetaminophen for 96-hours in two definitive concentrations-response assays with and without an exogenous MAS. Two static-renewal tests with acetaminophen and the MAS treated with carbon monoxide, cimetidine, ellipticine, diethyl maleate, and supplemented with glutathione were also performed. Addition of the MAS decreased the 96-hour LC50 and EC50 (malformation) values of unactivated acetaminophen 3.9-fold and 7.1-fold, respectively. Addition of the carbon monoxide- and ellipticine-inhibited MAS, as well as, the glutathione-supplemented MAS decreased the

developmental toxicity of activated acetaminophen to levels near that of the unactivated parent compound. Cimetidine-inhibited MAS also reduced the developmental toxicity of acetaminophen, but not to the extent observed with the carbon monoxide- and ellipticine-inhibited, or glutathione-supplemented MAS. Addition of the diethyl maleate-treated MAS substantially increased the developmental toxicity of acetaminophen. Results indicated that a highly reactive intermediate formed as the result of mixed function oxidase-mediated metabolism (possibly P-448) significantly increased the developmental toxicity of acetaminophen. Glutathione was also found to play a major role in intermediate detoxification *in vitro*.

In summary, the developmental toxicity of four test compounds (cyclophosphamide, coumarin, 2-acetylaminofluorene [2-AAF], and trichloroethylene) was evaluated using the traditional static-renewal method described in the ASTM Standard Guide for conducting FETAX (22) with minor modification. Differently-induced rat liver microsomes in the form of a test kit was co-cultured with FETAX in an identical fashion to that described in Experimental Methods Section. Results from these studies are provided in this report.

Body

Experimental Methods

Animal Care and Breeding

Xenopus adult care, breeding, and embryo collection were performed as described in the ASTM Standard Guide (22).

Metabolic Activation System(s)

The metabolic activation system consisted of post-isolation mixed sets of β -naphthoflavone, phenobarbital, and isoniazid-induced rat liver microsomes. Several studies including some in our own laboratories have demonstrated that the use of Aroclor 1254-induced microsomes with compounds metabolized by Aroclor 1254-repressed isozymes (pyrazole/isoniazid-induced P-450) was counter-productive, thus isoniazid was also been selected as a P-450 inducing agent. In addition, several chemicals have been shown to be metabolized by Aroclor 1254-repressible isozymes including: trichloroethylene, acetone, ethanol, and N-nitrosodimethylamine. Since the use of commercial preparations of rat liver microsomes, including both S-9 and lypholyzed purified preparations, has been unsuccessful due to a combination of high residual Aroclor 1254 levels and lipid peroxidation-induced toxicity, we purified rat liver S-9 supernatant. Purification of the rat liver microsomes was performed by the method of Kitchin and Woods (34). The pelleted microsomes were resuspended in cold 0.05 M Tris-HCl buffer and the protein content was determined. For each experiment performed the same amount of P-450 activity was added to the dishes even though the amount of protein varied. P-450 activity inferred by the amount of formaldehyde generated from the N-demethylation of aminopyrine and N-nitrosodimethylamine by the method of Nash (35). After quantification, the microsomes were split into four aliquots and

frozen in liquid nitrogen for use in each succeeding day of the experiment. Freezing of microsomes was determined to be an acceptable procedure (36). Randomly selected aliquots of the differently induced microsome mixes were treated with CO immediately prior to co-culture with the *Xenopus* embryos.

Assay Protocol

All FETAX tests were conducted in accordance with the methods described in the ASTM Standard Guide (22) with the minor modifications described below. For test conducted with unactivated toxicant (no MAS), groups of 20 embryos were placed in 60 mm plastic Petri dishes with varying concentrations of the toxicant. The test compounds were dissolved in FETAX solution, a reconstituted water media designed for the culture of *Xenopus* larvae (16). One percent DMSO (v/v) was used as a co-solvent as needed (4). Four separate dishes of 20 embryos were exposed to FETAX Solution alone and designated FETAX Solution controls. For each definitive test, 12-18 concentrations were tested in duplicate. Tests conducted with the MAS and the inhibited MAS were also conducted in duplicate with 20 embryos per replicate concentration. Each activated treatment received 0.04 U/ml of APD activity (expressed as μM formaldehyde generated/minute), a NADPH generating system, and 100 U/mL penicillin--100 U/mL streptomycin. The NADPHH generating system will consisted of 3.5 mM glucose-6-phosphate, 0.31 IU glucose-6-phosphate dehydrogenase, 0.1 mM NADP, and 0.7 μM NADPH. Controls for the MAS, CO inhibited-MAS, cyclophosphamide [FETAX reference proteratogen] (2), and unactivated toxicant were also be conducted with each individual test.

One range test, one definitive unactivated test, and two separate definitive tests with and without the MAS were conducted with each compound in the same format utilized during Phase III of the recent interlaboratory study (ILS) conducted with FETAX (29). Although limited testing was performed with the Aroclor 1254-induced MAS, previous ILS work (26-29) suggested that the most successful approach for consistent interlaboratory testing was to perform testing in the following manner: 1) initial range test to estimate 96-hour LC50 and EC50 (malformation) values, 2) definitive unactivated test (no MAS) using sliding scale to select concentrations (29), and 3) two definitive concentration-response experience tests with and without the exogenous MAS using the predicted EC5 (malformation) and LC5, EC16 and LC16, EC50 and LC50, EC84 and LC84, and EC95 and LC95 from the definitive unactivated test. This approach has been highly effective in testing performed since the completion of the ILS program (26-29). The pH of all stock solutions was strictly maintained between 7.0-7.2 if possible. Embryos were cultured at $23 \pm 1^\circ\text{C}$ throughout the test. All solutions will be removed every 24 hours of the 4-day test and fresh solutions will be added. Dead embryos were removed when solutions were changed. Following 96-hours of exposure, surviving embryos were fixed in 3.0% formalin, pH 7.0. The number of live-malformed embryos and the stage of development (37) were then be ascertained using a dissection microscope.

Data Analysis

Probit analysis (38) was used to determine the 96-hour median lethal concentration (96-hour LC50) the 96-hour EC50 (the concentration inducing gross terata in 50% of the surviving larvae) and their respective 95% fiducial intervals. Comparison of concentration-response data will be performed by the method of Sprague and Fogels (39). TI values (18) were determined for each test. Head to tail length will be measured as an indicator of embryo growth using Sigma Scan[®] digitizing software (Jandel Scientific, Corte Madera, CA) and a personal computer. Concentrations inducing growth inhibition (MCIG) were calculated using the Williams test (grouped, $P=0.05$).

Results and Discussion

Development of Exogenous Metabolic Activation System

Preliminary range finding results of tests with cyclophosphamide, coumarin, 2-AAF, and trichloroethylene, are provided in Table 1. Results from these unactivated definitive tests were used to generate the concentrations to be used in the evaluation of the differently induced MASs. As previously mentioned in the Experimental Methods section, tests with each of the four compounds with and without each of an Aroclor 1254-, phenobarbital-, β -naphthoflavone-, and isoniazid-induced MAS were performed concurrently. In addition, the use of post-isolation mixes of the phenobarbital-, β -naphthoflavone-, and isoniazid-induced MASs were prepared and tested concurrently with the other treatments.

Results of MAS performance evaluation with cyclophosphamide is provided in Table 2. Unactivated cyclophosphamide or cyclophosphamide co-cultured with the isoniazid-induced MAS were not developmentally toxic at concentrations $>4,000$ mg/L. In Test No. 1, Aroclor 1254-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to 1,500.0 and 590.0 mg/L, respectively. Phenobarbital-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to 1,700.0 and 610.0 mg/L, respectively. The β -naphthoflavone-induced MAS less effectively bioactivated cyclophosphamide to 96-hour LC50 and EC50 (malformation) values of 3,090.0 and 1,810.0 mg/L, respectively. As previously mentioned, the isoniazid-induced MAS was ineffective in bioactivating cyclophosphamide. The post-isolation mixture of the phenobarbital-, β -naphthoflavone-, and isoniazid-induced MASs was highly effective in bioactivating cyclophosphamide producing LC50 and EC50 (malformation) values of 1,340.0 and 400.0 mg/L, respectively. The mixed MAS also produced the greatest TI value of 3.4 and inhibited growth at comparable levels (100.0 mg/L) to the Aroclor 1254- and phenobarbital-induced MAS tests. Results from Test No. 2 were similar to results from Test No. 1 in which each of the Aroclor 1254-induced, phenobarbital-induced, and mixed MASs were successful in bioactivating cyclophosphamide, whereas, the β -naphthoflavone- and isoniazid-induced systems were less effective and ineffective in bioactivating cyclophosphamide, respectively. Bioactivated cyclophosphamide induced microencephaly, ophthalmic abnormalities, and severe skeletal kinking. These abnormalities were similar to those recorded in previous studies and similar to those identified in rodents, rabbits, and monkeys (1, 31). The results of the present study were not necessarily unexpected as cyclophosphamide has been shown to primarily bioactivated by

Table 1**Results of Unactivated Definitive FETAX Tests**

Compound	LC50¹	EC50^{1,2}	TI³	MCIG⁴
Cyclophosphamide	6760.0 (6650.0 - 6880.0)	4830.0 (4710.0 - 4950.0)	1.4	6000.0
Trichloroethylene	490.0 (480.0 - 500.0)	51.0 (49.0 - 53.0)	9.6	400.0
Coumarin	168.7 (154.2 - 186.2)	59.8 (58.5 - 61.1)	2.8	100.0
2-Acetylaminofluorene	110.0 (105.0 - 115.0)	30.0 (25.0 - 35.0)	3.7	90.0

¹ Expressed as mg/L.

² Malformation.

³ Teratogenic Index calculated by dividing the 4-d LC50/4-d EC50 (malformation).

⁴ Minimum Concentration to Inhibit Growth (Williams Test, P=0.05).

Table 2

**Results of FETAX tests with Cyclophosphamide and Differently-Induced
Exogenous Metabolic Activation Systems (MASs)**

Test No.	MAS	LC50 ¹	EC50 ^{1,2}	TI ³	MCIG ⁴
1	None	>4000.0 (ND)	>4000.0 (ND)	ND	>4000.0
	Aroclor 1254	1500.0 (1420.0-1580.0)	590.0 (510.0-680.0)	2.5	100.0
	Phenobarbital	1700.0 (1610.0-1810.0)	610.0 (530.0-700.0)	2.8	100.0
	β -Naphthoflavone	3090.0 (3000.0-3170.0)	1810.0 (1720.0-1910.0)	1.7	1000.0
	Isoniazid	>4000.0 (ND)	>4000.0 (ND)	ND	>4000.0
	Mixed ⁵	1340.0 (1260.0-1430.0)	400.0 (340.0-470.0)	3.4	100.0

(continued)

**Results of FETAX tests with Cyclophosphamide and Differently-Induced
Exogenous Metabolic Activation Systems (MASs)**

(continued)

Test No.	MAS	LC50 ¹	EC50 ^{1,2}	TI ³	MCIG ⁴
2	None	>4000.0 (ND)	>4000.0 (ND)	ND	>4000.0
	Aroclor 1254	1850.0 (1740.0-1980.0)	710.0 (620.0-800.0)	2.6	100.0
	Phenobarbital	1590.0 (1510.0-1670.0)	920.0 (870.0-970.0)	1.7	100.0
	β- Naphthoflavone	3130.0 (3000.0-3280.0)	1860.0 (1750.0-1970.0)	1.7	1000.0
	Isoniazid	>4000.0 (ND)	>4000.0 (ND)	ND	>4000.0
	Mixed ⁵	1690.0 (1580.0-1810.0)	720.0 (630.0-810.0)	2.4	100.0

¹ Expressed as mg/L.

² Malformation.

³ Teratogenic Index calculated by dividing the 4-d LC50/4-d EC50 (malformation).

⁴ Minimum Concentration to Inhibit Growth (Williams Test, P=0.05).

⁵ Consists of equal post-isolation activity mix of phenobarbital-, β-naphthoflavone-, and isoniazid-induced rat liver microsomes.

phenobarbital-inducible P-450, but with some cross specificity with aryl hydrocarbon hydroxylase isozymes (B-naphthoflavone-inducible P-450) (1,29).

Results of MAS performance evaluation with coumarin is provided in Table 3. Tests with unactivated coumarin produced 96-hour LC50 and EC50 (malformation), TI, and MCIG values of 129.4 mg/L, 49.0 mg/L, 2.6, and 50.0 mg/L. In Test No. 1, Aroclor 1254-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to 68.9 and 25.9 mg/L, respectively. Phenobarbital-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to a lesser extent to 93.3 and 51.5 mg/L, respectively. The β -naphthoflavone-induced MAS effectively bioactivated coumarin to 96-hour LC50 and EC50 (malformation) values of 57.2 and 16.9 mg/L, respectively. The isoniazid-induced MAS was also less effective in bioactivating coumarin producing LC50 and EC50 (malformation) values of 98.0 and 46.1 mg/L. The post-isolation mixture of the phenobarbital-, B-naphthoflavone-, and isoniazid-induced MASs was highly effective in bioactivating coumarin producing LC50 and EC50 (malformation) values of 37.3 and 17.3 mg/L, respectively. Each of the MASs did not appreciably alter the TI values. The β -naphthoflavone-induced MAS inhibited growth at 10.0 mg/L, whereas, the Aroclor 1254-induced and mixed MASs inhibited growth at 25.0 mg/L. The isoniazid- and phenobarbital-induced MASs tests did not appreciably alter growth compared to unactivated coumarin. Results from Test No. 2 were similar to results from Test No. 1 in which each of the Aroclor 1254-induced, β -naphthoflavone-induced, and mixed MASs were successful in bioactivating coumarin, whereas, the phenobarbital- and isoniazid-induced systems were less effective and ineffective in bioactivating coumarin, respectively. Bioactivated coumarin induced microphthalmia, craniofacial abnormalities, visceral edema, and mouth defects. These abnormalities were similar to those recorded in previous studies and similar to those identified in rodents and rabbits, however somewhat more controversial than cyclophosphamide (40). As with cyclophosphamide, the results of the present study were not necessarily unexpected as coumarin has been shown to primarily bioactivated by aryl hydrocarbon hydroxylase isozymes (B-naphthoflavone-inducible P-450) (41).

Results of MAS performance evaluation studies with 2-AAF are provided in Table 4. Tests with unactivated 2-AAF produced 96-hour LC50 and EC50 (malformation), TI, and MCIG values of 125.9 mg/L, 8.7 mg/L, 14.5, and 125.0 mg/L. In Test No. 1, Aroclor 1254-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to 17.9 and 7.0 mg/L, respectively. Phenobarbital-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to a lesser extent to 75.4 and 12.1 mg/L, respectively. The β -naphthoflavone-induced MAS effectively bioactivated 2-AAF to 96-hour LC50 and EC50 (malformation) values of 17.4 and 7.2 mg/L, respectively. The isoniazid-induced MAS was also less effective in bioactivating 2-AAF producing LC50 and EC50 (malformation) values of 100.2 and 10.7 mg/L. The post-isolation mixture of the phenobarbital-, β -naphthoflavone-, and isoniazid-induced MASs was highly effective in bioactivating 2-AAF producing LC50 and EC50 (malformation) values of 17.7 and 7.2 mg/L, respectively. Each of the MASs, with the exception of the isoniazid-induced MAS appreciably reduced the TI value of unactivated 2-AAF. The B-naphthoflavone-induced, Aroclor 1254-induced, and mixed MAS inhibited growth at 10.0 mg/L. The phenobarbital- and isoniazid-induced MAS tests inhibited growth at 50.0 and 100.0 mg/L, respectively. Results from Test No. 2 were similar to results from Test No. 1 in which each of the Aroclor 1254-

Table 3**Results of FETAX tests with Coumarin and Differently-Induced Exogenous Metabolic Activation Systems (MASs)**

Test No.	MAS	LC50 ¹	EC50 ^{1,2}	TI ³	MCIG ⁴
1	None	129.4 (127.2-131.6)	49.0 (48.1-55.8)	2.6	50.0
	Aroclor 1254	68.9 (62.6-75.8)	25.9 (23.1-29.1)	2.7	25.0
	Phenobarbital	93.3 (90.7-95.9)	51.5 (43.1-61.6)	1.8	50.0
	β -Naphthoflavone	57.2 (51.8-63.2)	16.9 (15.0-19.6)	3.4	10.0
	Isoniazid	98.0 (94.6-101.5)	46.1 (43.2-51.2)	2.1	50.0
	Mixed ⁵	37.3 (32.4-43.0)	17.1 (15.0-19.6)	2.2	25.0

(continued)

Results of FETAX tests with Coumarin and Differently-Induced Exogenous Metabolic Activation Systems (MASS)

(continued)

Test No.	MAS	LC50 ¹	EC50 ^{1,2}	TI ³	MCIG ⁴
2	None	136.8 (130.6-143.2)	43.0 (38.0-47.1)	3.2	50.0
	Aroclor 1254	73.5 (68.4-79.0)	34.8 (31.0-39.1)	2.1	25.0
	Phenobarbital	94.8 (91.8-97.9)	53.2 (47.4-59.7)	1.8	50.0
	β- Naphthoflavone	64.0 (59.4-70.0)	19.7 (16.7-23.3)	3.3	10.0
	Isoniazid	106.6 (101.9-111.5)	48.4 (43.7-53.5)	2.2	50.0
	Mixed ⁵	39.8 (35.1-45.1)	20.9 (18.5-23.4)	1.9	25.0

¹ Expressed as mg/L.

² Malformation.

³ Teratogenic Index calculated by dividing the 4-d LC50/4-d EC50 (malformation).

⁴ Minimum Concentration to Inhibit Growth (Williams Test, P=0.05).

⁵ Consists of equal post-isolation activity mix of phenobarbital-, β-naphthoflavone-, and isoniazid-induced rat liver microsomes.

Table 4**Results of FETAX tests with 2-Acetylaminofluorene and Differently-Induced Exogenous Metabolic Activation Systems (MASs)**

Test No.	MAS	LC50 ¹	EC50 ^{1,2}	TI ³	MCIG ⁴
1	None	125.9 (120.8-131.8)	8.7 (6.4-8.0)	14.5	125.0
	Aroclor 1254	17.9 (17.4-18.4)	7.0 (6.1-8.0)	2.6	10.0
	Phenobarbital	75.4 (72.3-78.6)	12.1 (10.5-13.9)	6.2	50.0
	β -Naphthoflavone	17.4 (16.7-18.3)	7.2 (6.4-8.0)	2.4	10.0
	Isoniazid	100.2 (93.7-107.1)	10.7 (9.4-12.3)	9.4	100.0
	Mixed ⁵	17.7 (15.9-19.7)	7.5 (6.5-8.6)	2.4	10.0

Table 4 (continued)**Results of FETAX tests with 2-Acetylaminofluorene and Differently-Induced Exogenous Metabolic Activation Systems (MASs)**

Test No.	MAS	LC50 ¹	EC50 ^{1,2}	TI ³	MCIG ⁴
2	None	130.7 (126.5-135.0)	9.1 (8.2-10.1)	14.4	125.0
	Aroclor 1254	22.0 (20.4-23.6)	6.4 (5.5-7.4)	3.4	10.0
	Phenobarbital	78.7 (73.5-84.2)	28.1 (15.0-21.5)	4.3	50.0
	β -Naphthoflavone	21.0 (19.3-22.9)	6.7 (5.9-7.7)	3.1	10.0
	Isoniazid	90.8 (84.5-97.6)	14.2 (12.3-16.4)	6.4	100.0
	Mixed ⁵	18.0 (15.8-20.5)	7.1 (6.4-7.9)	2.5	10.0

¹ Expressed as mg/L.² Malformation.³ Teratogenic Index calculated by dividing the 4-d LC50/4-d EC50 (malformation).⁴ Minimum Concentration to Inhibit Growth (William Test, P=0.05).⁵ Consists of equal post-isolation activity mix of phenobarbital-, β -naphthoflavone-, and isoniazid-induced rat liver microsomes.

induced, β -naphthoflavone-induced, and mixed MASs were successful in bioactivating 2-AAF, whereas, the phenobarbital- and isoniazid-induced systems were less effective and ineffective in bioactivating 2-AAF, respectively. Bioactivated 2-AAF induced pericardial edema, craniofacial mal-development, skeletal kinking, and microencephaly. These abnormalities were similar to those recorded in previous FETAX studies and similar to those identified in rodents and rabbits (3). As with cyclophosphamide, the results of the present study were not necessarily unexpected as 2-AAF has been shown to primarily bioactivated by aryl hydrocarbon hydroxylase isozymes (β -naphthoflavone-inducible P-450) (42).

Results of MAS performance evaluation with trichloroethylene is provided in Table 5. Unactivated trichloroethylene induced 96-hour LC50, EC50 (malformation), TI, and MCIG values of 412.0 mg/L, 40.9 mg/L, 10.1, and 152.0 mg/L. In Test No. 1, the Aroclor 1254-induced MAS and the β -naphthoflavone-induced MASs each did not appreciably alter the 96-hour LC50, EC50 (malformation), TI, and MCIG values. The phenobarbital-induced MAS only altered the 96-hour EC50 (malformation) by reducing it to 18.0 mg/L. The isoniazid-induced MAS reduced the 96-hour LC50 and EC50 (malformation) values to 190.4 and 11.9 mg/L, respectively. The TI value was increased to 16.0. The post-isolation mixture of the phenobarbital-, β -naphthoflavone-, and isoniazid-induced MASs was highly effective in bioactivating trichloroethylene producing LC50 and EC50 (malformation) values of 181.3 and 7.5 mg/L, respectively. The mixed MAS also produced the greatest TI value of 24.2 and inhibited growth at comparable levels (50.0 mg/L) to those observed with isoniazid-induced MAS tests. Results from Test No. 2 were similar to results from Test No. 1 in which each of the isoniazid-induced and mixed MASs were successful in bioactivating trichloroethylene, whereas, the β -naphthoflavone-, Aroclor 1254-, and to a lesser extent the phenobarbital-induced systems were less effective in bioactivating trichloroethylene. Bioactivated trichloroethylene induced mal-developed guts, microencephaly, hydroencephaly, hypognathia, microphthalmia, abnormal mouth development, and muscular kinking. These abnormalities were similar to those recorded in previous studies and similar to those identified in rodents, rabbits, and monkeys (6). The results of the present study were not necessarily unexpected as trichloroethylene has been shown to primarily bioactivated by isoniazid-inducible P-450 (43).

Raw data from the FETAX testing is provided as Appendix A.

Development of Metabolic Action System Test Kits

In order to facilitate the use of a simplistic metabolic activation system to use outside of our laboratory which would benefit the U.S. Army, and the scientific community, a MAS test kit was developed and evaluated in the aforementioned studies. The MAS test kit was developed and refined during Phase I studies and is proposed for use in the interlaboratory validation study which encompasses Phase II work. A model of the MAS test kit is included as Appendix B.

Table 5**Results of FETAX tests with Trichloroethylene and Differently-Induced Exogenous Metabolic Activation Systems (MASs)**

Test No.	MAS	LC50 ¹	EC50 ^{1,2}	TI ³	MCIG ⁴
1	None	412.0 (370.4-458.3)	40.9 (35.4-42.3)	10.1	152.0
	Aroclor 1254	378.1 (339.5-421.4)	40.2 (34.6-46.6)	9.4	152.0
	Phenobarbital	412.1 (347.67-437.75)	18.0 (14.6-22.3)	22.9	152.0
	β -Naphthoflavone	390.1 (349.7-437.8)	39.7 (34.0-46.3)	9.8	152.0
	Isoniazid	190.4 (167.3-216.6)	11.9 (8.5-16.7)	16.0	50.0
	Mixed ⁵	181.3 (158.8-206.9)	7.5 (6.5-8.7)	24.2	50.0

(continued)

Table 5 (continued)

**Results of FETAX tests with Trichloroethylene and Differently-Induced
Exogenous Metabolic Activation Systems (MASs)**

Test No.	MAS	LC50 ¹	EC50 ²	TI ³	MCIG ⁴
2	None	426.0 (384.5-472.9)	36.2 (30.8-42.6)	11.8	152.0
	Aroclor 1254	383.5 (341.6-430.5)	37.3 (32.0-43.4)	10.3	152.0
	Phenobarbital	410.8 (367.17-459.6)	19.5 (16.4-23.1)	21.1	97.0
	β -Naphthoflavone	388.9 (344.6-438.9)	40.2 (34.2-47.3)	9.7	152.0
	Isoniazid	175.3 (153.4-200.2)	11.5 (8.7-15.2)	15.2	50.0
	Mixed ⁵	169.1 (147.8-193.5)	7.6 (6.4-9.1)	22.3	50.0

¹ Expressed as mg/L.

² Malformation.

³ Teratogenic Index calculated by dividing the 4-d LC50/4-d EC50 (malformation).

⁴ Minimum Concentration to Inhibit Growth (Williams Test, P=0.05).

⁵ Consists of equal post-isolation activity mix of phenobarbital-, β -naphthoflavone-, and isoniazid-induced rat liver microsomes.

Conclusions

Results from these studies suggested that the Aroclor 1254-induced MAS could effectively be replaced by a mixed lot of phenobarbital-, β -naphthoflavone-, and isoniazid-induced rat liver microsomes. In addition, the use of a MAS test kit is a practicable means of facilitating external use of the system outside of our laboratory, particularly by the U.S. Army. Overall, the results from these studies suggested that continued validation of an exogenous metabolic activation system for FETAX is warranted in Phase II SBIR Studies to increase the overall predictability of the assay, and thus acceptance by the scientific and regulatory communities.

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2. D.J. Fort, T.L. Propst, B.C. Faulkner, E.L. Stover, J.A. Bantle, and R.A. Finch. Utilizing differently induced and inhibited rat liver microsomes to evaluate modes of biotransformation and detoxification, J. Appl. Toxicol., in preparation.

Abstracts and Presentations

1. D.J. Fort. Applications of FETAX, NIEHS FETAX Workshop, RTP, NC, April 1997.
2. D.J. Fort, T. Propst, and E.L. Stover. Evaluating Mechanisms of Action of Development Toxicants Using FETAX as a Model, Society of Toxicology, Cincinnati, OH, March 1997.
3. D.J. Fort, T. Propst, and E.L. Stover. Evaluating Biotransformation of Development Toxicants Using FETAX as a Model, Teratology Society, Palm Beach, FL, June 1997.
4. D.J. Fort, E.L. Stover, T. Propst, B. Faulkner, J.A. Bantle, and R. Finch. Validation of a Whole-Embryo Limb Development Assay Using *Xenopus laevis*. Society of Environment Toxicology and Chemistry (SETAC), San Francisco, CA, November 1997.

Project Personnel

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Kendall W. King, P.E.
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Appendix A

Raw Data

FETAX SUMMARY SHEET

Test No. 1

Test Material <u>Conmarin</u>	Investigator <u>Font</u>
Source <u>US Army SER</u>	Lab <u>SA</u>
CAS No. _____ Lot No. _____	Test Start Date _____
Composition/Purity _____	Test End Date _____
Solvent _____ Conc. _____	Test Units (i.e., mg/ml) <u>mg/L</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>— pH —</u>					
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
_____ X 100 = %		
Total Number	<u>0</u> : <u>80</u> X 100 = <u>0</u> %	<u>0</u> : <u>80</u> X 100 = <u>0</u> %
Solvent Control	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length _____ mm	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG)		

UNMUTATED/BNF/INIT/MIX/Arach/PB
 TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL		93.32	51.49
LC ₅₀ 129.36/57.19/98.0/37.33/68.87	EC ₅₀ 49.01/16.94/46.13/47.14/25.89/		
95% Confidence limits 94.64-101.49/32.43-42.97	95% Confidence Limits 48.08-55.71/14.89-19.27/		
127.19-131.56/51.75-63.20 62.61-75.15	48.18-51.70/14.97-19.62/23.07-29.05/		

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀)

43.06-61.58

90.72-95.92

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

FETAX MORTALITY DATA

Investigator	Fast	Test Material	COMMAN
Date		Test Number	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
FX							0	0	
FX							0	0	
MAS									
5							0	0	
20							0	0	
27							0	0	
60							0	0	
86							0	0	
100							0	0	
115							0	0	
150							1	2	3
200							20	20	40
5							20	20	40
20							0	0	0
27							0	0	0
60							3	2	5
↓							5	6	11
5							20	20	40
20							0	0	0
27							0	0	0
60							0	0	0
86							0	0	0
100							3	2	5
115							10	6	16
↓							20	20	40

unad.

PMF

INT

Investigator	Date	Test Material	Test Number						
		Coumarin							
Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
5							0 : 0	0	
20							3 : 1	4	
27							7 : 5	12	
60							11 : 17	28	
↓							20 : 20	40	
5							0 : 0	0	
20							0 : 0	0	
27							1 : 2	3	
60							5 : 3	8	
86							10 : 12	22	
100							20 : 20	40	
115							20 : 20	40	
5							0 : 0	0	
20							↓ ↓	0	
27							↓ ↓	0	
60							↓ ↓	0	
86							2 : 1	3	
100							20 : 13	33	
115							20 : 20	40	

PB

FETAX MALFORMATION DATA

Investigator	Font	Test Material	Cammarin
Date		Test Number	

MALFORMATION	CONCENTRATION																			MIX			TOTAL				
	FX	FX	E	20	27	60	86	100	115	5	20	27	60	86	100	115	5	20	27	60							
Severe	0	0	0	2	10	24	40	40	37	0	15	35	29	0	0	0	8	23	35	24	-	0	13	28	12		
Stunted	40	40	40						37	40	40	35	29	40									40	36	28	12	
Gut																											
Edema																											
Multiple																											
Cardiac																											
Abdominal																											
Facial																											
Cephalic																											
Optic																											
Tail																											
Notochord																											
Fin																											
Face																											
Eye																											
Brain																											
Hemorrhage																											
Cardiac																											
Blisters																											
Other-specify																											
No. Malformed	0	0	0	2	10	24	40	40	37	0	15	35	29	0	0	0	8	23	35	24	-	0	13	28	12		
Total No.	40	40	40						37	40	40	35	29	40								40	36	28	12		
Comments:																											

Comments:

FETAX MALFORMATION DATA

Investigator	Test Material
Date	Test Number

MALFORMATION	CONCENTRATION										TOTAL
	5	20	27	60	86	27	40	86	100		
Severe											
Stunted											
Gut											
Edema											
Multiple											
Cardiac											
Abdominal											
Facial											
Cephalic											
Optic											
Tail											
Notochord											
Fln											
Face											
Eye											
Brain											
Hemorrhage											
Cardiac											
Blisters											
Other-specify											
No. Malformed	0	4	26	32	18	8	22	19	7		
Total No.	40	40	40	32	18	40	40	37	7		

5 20
0 0
40 40

Comments:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 1/30/97
 TEST NUMBER: 1
 DURATION: 4 D
 CHEMICAL: COUMARIN UNACT.
 SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	100.00	115.00	150.00	.00	.00	.00	.00	.00	.00
NUMBER EXPOSED:	40	40	40	0	0	0	0	0	0
MORTALITIES:	0	3	40	0	0	0	0	0	0
SPEARMAN-KARBER TRIM:	.00%								

SPEARMAN-KARBER ESTIMATES: LC50: 129.36
 VAR OF LN OF EST. : .71284D-04
 95% LOWER CONFIDENCE: 127.19
 95% UPPER CONFIDENCE: 131.56

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
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 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 1/30/97
 TEST NUMBER: 1
 DURATION: 4 D
 CHEMICAL: COUMARIN
 SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	20.00	27.00	60.00	86.00	.00	.00	.00	.00	.00
NUMBER EXPOSED:	40	40	40	40	0	0	0	0	0
MORTALITIES:	0	5	11	40	0	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 57.19
VAR OF LN OF EST. : .24975D-02
95% LOWER CONFIDENCE: 51.75
95% UPPER CONFIDENCE: 63.20

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TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

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TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/30/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN INH
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	60.00	86.00	100.00	115.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	5	16	40	0	0	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: LC50: 98.00
VAR OF LN OF EST. : .30504D-03
95% LOWER CONFIDENCE: 94.64
95% UPPER CONFIDENCE: 101.49

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/30/97
TEST NUMBER: 1
DURATION: 4 D

CHEMICAL: COUMARIN
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	4	12	28	40	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 37.33
VAR OF LN OF EST. : .49454D-02
95% LOWER CONFIDENCE: 32.43
95% UPPER CONFIDENCE: 42.97

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/30/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN AROCLOR
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	20.00	27.00	60.00	86.00	100.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	3	8	22	40	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 68.87
VAR OF LN OF EST. : .22691D-02
95% LOWER CONFIDENCE: 62.61
95% UPPER CONFIDENCE: 75.75

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 1/30/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN PB
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	60.00	86.00	100.00	115.00	100.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	3	33	40	40	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 93.32
VAR OF LN OF EST. : .18934D-03
95% LOWER CONFIDENCE: 90.79
95% UPPER CONFIDENCE: 95.92

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 1/31/97
 TEST NUMBER: 1
 DURATION: 4 D
 CHEMICAL: COUMARIN
 SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	
MORTALITIES:	0	2	10	14	40	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 49.01
 VAR OF LN OF EST. : .41671D-02
 95% LOWER CONFIDENCE: 43.08
 95% UPPER CONFIDENCE: 55.77

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 1/31/97
 TEST NUMBER: 1
 DURATION: 4 D
 CHEMICAL: COUMARIN BNF
 SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	35	29	40	0	0	
MORTALITIES:	0	15	35	29	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: 16.94
VAR OF LN OF EST. : .41659D-02
95% LOWER CONFIDENCE: 14.89
95% UPPER CONFIDENCE: 19.27

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN INH
SPECIES: FETAX

RAW DATA:

CONCENTRATION(4)	20.00	27.00	60.00	86.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	35	40	0	0	0
MORTALITIES:	0	8	23	35	40	0	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: EC50: 46.13
VAR OF LN OF EST. : .32569D-02
95% LOWER CONFIDENCE: 41.15
95% UPPER CONFIDENCE: 51.70

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 1
DURATION: 4 D

CHEMICAL: COUMARIN MIX
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	36	28	12	40	0	0	0
MORTALITIES:	0	3	16	12	40	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 27.41
VAR OF LN OF EST. : .41478D-02
95% LOWER CONFIDENCE: 24.10
95% UPPER CONFIDENCE: 31.18

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN
SPECIES: FETAX MIX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	36	28	32	18	0	0	0
MORTALITIES:	0	13	28	24	18	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 17.14
VAR OF LN OF EST. : .45564D-02
95% LOWER CONFIDENCE: 14.97
95% UPPER CONFIDENCE: 19.62

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	32	18	0	0	
MORTALITIES:	0	4	26	32	18	0	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: EC50: 25.89
VAR OF LN OF EST. : .33158D-02
95% LOWER CONFIDENCE: 23.07
95% UPPER CONFIDENCE: 29.05

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN PB
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	27.00	60.00	86.00	100.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	37	7	18	0	0	
MORTALITIES:	8	22	29	7	18	0	0	0
SPEARMAN-KARBER TRIM:				20.00%				

SPEARMAN-KARBER ESTIMATES: EC50: 51.49
VAR OF LN OF EST. : .80018D-02
95% LOWER CONFIDENCE: 43.06
95% UPPER CONFIDENCE: 61.58

FETAX SUMMARY SHEET

Test No. 2

Test Material <u>Coumarin</u>	Investigator <u>Font</u>
Source <u>US Army SBR</u>	Lab <u>SA</u>
CAS No. _____ Lot No. _____	Test Start Date _____
Composition/Purity _____	Test End Date _____
Solvent _____ Conc. _____	Test Units (i.e., mg/ml) <u>mg/L</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %		
	2 : 80 X 100 = 2.5%	_____ : _____ X 100 = _____%
Solvent Control	_____ : _____ X 100 = _____%	_____ : _____ X 100 = _____%
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL		94.77	
LC ₅₀ 136.78/64.02/106.60/39.80/73.53	EC ₅₀ 42.29/19.71/48.35/20.89/34.84/53.17		
95% Confidence Limits 35.11-45.11/68.42-79.24	95% Confidence Limits 18.50-23.40/31.02-39.13		
130.63-143.23/69.41-68.99/101.89-111.52	37.99-47.08/16.65-23.33/43.71-53.49		
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			
91.75-97.89			
47.35-59.72			

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ X 100 = _____%	_____ : _____ X 100 = _____%
2500 mg/L	_____ : _____ X 100 = _____%	_____ : _____ X 100 = _____%

[illegible]

Arvold

22

FETAX MALFORMATION DATA

Investigator	Font	Test Material	COMMAVIN
Date		Test Number	2

MALFORMATION	UNACT										CONCENTRATION										INH										TOTAL								
	F	F	5	20	27	60	86	100	115	150	5	20	27	60	86	27	60	86	100	115	5	20	27	60	86	100	115	5	20	27		60	86	100	115	5	20		
Severe																																							
Stunted																																							
Gut																																							
Edema																																							
Multiple																																							
Cardiac																																							
Abdominal																																							
Facial																																							
Cephalic																																							
Optic																																							
Tail																																							
Notochord																																							
Fin																																							
Face																																							
Eye																																							
Brain																																							
Hemorrhage																																							
Cardiac																																							
Blisters																																							
Other-specify																																							
No. Malformed	1	0	0	0	8	29	40	40	33	11	2	13	30	33	—	3	25	34	29	13	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
Total No.	40	38	40	40	40	40	40	40	33	11	40	40	39	33	—	40	40	35	29	13	40	40	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
Comments:																																							

Comments:

Investigator	Font	Test Material	Conmarin
Date		Test Number	2

MALFORMATION	MIX			Acror			CONCENTRATION										TOTAL
	5	20	27	60	5	20	27	60	86	5	20	27	60	86	100		
Severe																	
Stunted																	
Gut																	
Edema																	
Multiple																	
Cardiac																	
Abdominal																	
Facial																	
Cephalic																	
Optic																	
Tail																	
Notochord																	
Fin																	
Face																	
Eye																	
Brain																	
Hemorrhage																	
Cardiac																	
Blisters																	
Other-specify																	
No. Malformed	0	7	25	13	0	1	15	20	15	0	0	6	19	28	13		
Total No.	40	39	27	13	40	40	40	35	15	40	40	40	40	36	13		
Comments:																	

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.

TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN

LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.

ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 1/31/97

TEST NUMBER: 1

DURATION: 4 D

CHEMICAL: COUMARIN PB

SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/L)	20.00	27.00	60.00	86.00	100.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	36	13	0	0	0
MORTALITIES:	0	6	19	28	13	0	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: EC50: 53.17

VAR OF LN OF EST. : .33669D-02

95% LOWER CONFIDENCE: 47.35

95% UPPER CONFIDENCE: 59.72

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.

TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.

ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 1/31/97

TEST NUMBER: 2

DURATION: 4 D

CHEMICAL: COUMARIN MIX

SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/L)	5.00	20.00	27.00	60.00	.00	.00	.00	.
NUMBER EXPOSED:	40	39	27	13	0	0	0	0
MORTALITIES:	0	7	25	13	0	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 20.80

VAR OF LN OF EST. : .34513D-02

95% LOWER CONFIDENCE: 18.50

95% UPPER CONFIDENCE: 23.40

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.

TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.

ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 1/31/96

TEST NUMBER: 2

DURATION: 4 D

CHEMICAL: COUMARIN AROCLOR

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	35	15	0	0	0
MORTALITIES:	0	1	15	30	15	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 34.84

VAR OF LN OF EST. : .33751D-02

95% LOWER CONFIDENCE: 31.02

95% UPPER CONFIDENCE: 39.13

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.

TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.

ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 1/31/97

TEST NUMBER: 2

DURATION: 4 D

CHEMICAL: COUMARIN UNACT.

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	0	8	29	40	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 42.29

VAR OF LN OF EST. : .28794D-02

95% LOWER CONFIDENCE: 37.99

95% UPPER CONFIDENCE: 47.08

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.

TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.

ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 1/31/97

TEST NUMBER: 2

DURATION: 4 D

CHEMICAL: COUMARIN BNF

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	39	33	40	0	0	0
MORTALITIES:	2	13	30	33	40	0	0	0

SPEARMAN-KARBER TRIM: 5.00%

SPEARMAN-KARBER ESTIMATES: EC50: 19.71
VAR OF LN OF EST. : .71002D-02
95% LOWER CONFIDENCE: 16.65
95% UPPER CONFIDENCE: 23.33

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: COUMARIN INH
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	20.00	27.00	60.00	86.00	100.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	35	29	0	0	0
MORTALITIES:	0	3	25	34	29	0	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: EC50: 48.35
VAR OF LN OF EST. : .25411D-02
95% LOWER CONFIDENCE: 43.71
95% UPPER CONFIDENCE: 53.48

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 1/31/97
 TEST NUMBER: 2
 DURATION: 4 D
 CHEMICAL: COUMARIN
 SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	100.00	115.00	150.00	200.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	18	0	0	0	
MORTALITIES:	0	7	29	18	0	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 136.78
 VAR OF LN OF EST. : .52994D-03
 95% LOWER CONFIDENCE: 130.63
 95% UPPER CONFIDENCE: 143.23

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 1/31/97
 TEST NUMBER: 2
 DURATION: 4 D
 CHEMICAL: COUMARIN
 SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	20.00	27.00	60.00	86.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	18	0	0	0	
MORTALITIES:	0	1	7	18	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 64.02
VAR OF LN OF EST. : .13949D-02
95% LOWER CONFIDENCE: 59.41
95% UPPER CONFIDENCE: 68.99

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: FETAX
SPECIES: COUMARIN

RAW DATA:

CONCENTRATION(MG/L)	60.00	86.00	100.00	115.00	150.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	
MORTALITIES:	0	5	11	27	40	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 106.60
VAR OF LN OF EST. : .50901D-03
95% LOWER CONFIDENCE: 101.89
95% UPPER CONFIDENCE: 111.52

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 1
DURATION: 4 D

CHEMICAL: COUMARIN
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	20.00	27.00	60.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	1	13	27	40	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 39.80
VAR OF LN OF EST. : .39283D-02
95% LOWER CONFIDENCE: 35.11
95% UPPER CONFIDENCE: 45.11

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: FETAX
SPECIES: COUMARIN

RAW DATA:

CONCENTRATION(MG/L)	27.00	60.00	86.00	100.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	5	25	40	40	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 73.53
VAR OF LN OF EST. : .12997D-02
95% LOWER CONFIDENCE: 68.42
95% UPPER CONFIDENCE: 79.03

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 1/31/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: COUMARIN
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	60.00	86.00	100.00	115.00	86.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	4	27	40	40	0	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: LC50: 94.77
VAR OF LN OF EST. : .26255D-03
95% LOWER CONFIDENCE: 91.75
95% UPPER CONFIDENCE: 97.89

FETAX SUMMARY SHEET

Test No. 1

Test Material	2-AAF	Investigator	Font
Source	US Army SBIR	Lab	Stover
CAS No.		Lot No.	
Composition/Purity		Test Start Date	1/21/97
		Test End Date	1/25/97
Solvent		Conc.	
		Test Units (i.e., mg/ml)	mg/L

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock					
Control					
Highest Conc.					

FETAX CONTROL		MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed	X 100 = %		
Total Number		0 : 80 X 100 = 0 %	0 : 80 X 100 = 0 %
Solvent Control		_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length mm		Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)			

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL		17.70 /	
LC ₅₀ 125.90/75.38/17.41/100.21/17.91/		EC ₅₀ 8.66/12.06/7.16/10.74/6.98/7.48	
95% Confidence limits 120.26-131.81/72.33-78.55 16.60-18.25/93.73-107.13/17.41-18.42/		95% Confidence Limits 7.30-10.28/10.47-13.89 6.41-8.01/9.39-12.28/6.11-7.96/6.48-8.64	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			
15.90-19.69/			

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

Investigator	Fant	Test Material	2-AAF
Date	1/28/97	Test Number	1
Concentration			

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
FETAX							0	0	
FETAX							0	0	
MAS - PB							0	0	
CP+ PB							20	20	
CP- PB							0	0	
3							0	0	
9.6							0	0	
11							0	0	
30							0	0	
35							0	0	
50							10	0	
57							0	0	
110							12	3	
125							20	20	
150							20	20	
3							20	20	
9.6							0	0	
11							0	0	
30							1	2	
↓							20	20	
3							20	20	
9.6							0	0	
11							0	0	
30							0	0	
35							0	0	
50							0	0	
57							0	0	
110							0	0	
125							0	0	
150							0	0	
3							0	0	
9.6							0	0	
11							0	0	
30							0	0	
35							0	0	

0	0	0	3	13	40
0	0	0	3	7	20
0	0	0	3	6	20

35
50
57
110
125

98

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FETAX MORTALITY DATA

Investigator	Test Material
Date	Test Number

2-22-77

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
3							0	0	
9.6							0	0	
11							1	0	
30							40	20	
↓							20	20	
3							0	0	
9.6							1	1	
11							15	2	
30							17	16	
35							19	19	
50							20	20	
57							20	20	
↓							20	20	
3							0	0	
9.6							2	2	
11							3	3	
30							5	5	
35							5	5	
50							0	0	
57							3	2	
110							5	4	
125							20	20	
150							40	40	

Anchor

MIX

UNACT

Investigator		Test Material	2-AAF
Date		Test Number	2

MALFORMATION	Archer			M/X			CONCENTRATION										TOTAL	
	3	7.6	11	3	7.6	11	30	35	3	9.6	11	30	35	50	57	110		125
Severe	3	7.6	11	3	7.6	11	30	35	3	9.6	11	30	35	50	57	110	125	150
Stunted																		
Gut																		
Edema																		
Multiple																		
Cardiac																		
Abdominal																		
Facial																		
Cephalic																		
Optic																		
Tail																		
Notochord																		
Fin																		
Face																		
Eye																		
Brain																		
Hemorrhage																		
Cardiac																		
Blisters																		
Other-specify																		
No. Malformed	3	23	39	2	21	18	6	3	1	11	19	39	40	40	35	31	—	—
Total No.	40	46	39	40	38	33	6	3	40	—	—	—	—	—	40	35	31	—
Comments:																		

FETAX MALFORMATION DATA

Investigator	Fent	Test Material	2-APF
Date		Test Number	1

MALFORMATION	CONCENTRATION																			TOTAL			
	PB																						
	F	F	M	UR	3	9.6	11	30	35	50	57	3	9.6	11	3	9.6	11	30	35	50	57	110	
Severe																							
Stunted																							
Gut																							
Edema																							
Multiple																							
Cardiac																							
Abdominal																							
Facial																							
Cephalic																							
Optic																							
Tail																							
Notochord																							
Fln																							
Face																							
Eye																							
Brain																							
Hemorrhage																							
Cardiac																							
Blisters																							
Other-specify																							
No. Malformed	0	0	0	5	3	5	23	40	40	40	35	1	22	37	1	10	25	40	40	40	37	27	
Total No.	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	37	27	
Comments:																							

Comments:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

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LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: FETAX
SPECIES: 2-AAF

RAW DATA:

CONCENTRATION(MG/L)	57.00	110.00	125.00	150.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	5	9	40	0	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 125.90
VAR OF LN OF EST. : .52637D-03
95% LOWER CONFIDENCE: 120.26
95% UPPER CONFIDENCE: 131.81

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 2/3/97
 TEST NUMBER: 1
 DURATION: 4 D
 CHEMICAL: 2-AAF PB
 SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	50.00	57.00	110.00	150.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	5	40	40	0	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 75.38
 VAR OF LN OF EST. : .42497D-03
 95% LOWER CONFIDENCE: 72.33
 95% UPPER CONFIDENCE: 78.55

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 2/3/97
 TEST NUMBER: 1
 DURATION: 4 D
 CHEMICAL: 2-AAF
 SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	9.60	11.00	30.00	150.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	3	40	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 17.41
VAR OF LN OF EST. : .56294D-03
95% LOWER CONFIDENCE: 16.60
95% UPPER CONFIDENCE: 18.25

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF INH
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	50.00	57.00	110.00	125.00	110.00	125.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	0	3	13	40	39	40	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: LC50: 100.21
VAR OF LN OF EST. : .11150D-02
95% LOWER CONFIDENCE: 93.73
95% UPPER CONFIDENCE: 107.13

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D

CHEMICAL: 2-AAF AROCLOR
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	9.60	11.00	30.00	125.00	110.00	125.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0
MORTALITIES:	0	1	40	40	39	40	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: LC50: 17.91
VAR OF LN OF EST. : .19779D-03
95% LOWER CONFIDENCE: 17.41
95% UPPER CONFIDENCE: 18.42

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.

TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN

LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.

ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 2/3/97

TEST NUMBER: 1

DURATION: 4 D

CHEMICAL: 2-AAF MIX

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	
MORTALITIES:	0	2	7	34	37	40	C	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: LC50: 17.70

VAR OF LN OF EST. : .28534D-02

95% LOWER CONFIDENCE: 15.90

95% UPPER CONFIDENCE: 19.69

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 2/3/97
 TEST NUMBER: 1
 DURATION: 4 D
 CHEMICAL: 2-AAF UNACT.
 SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	
MORTALITIES:	1	11	19	39	40	40	0	0
SPEARMAN-KARBER TRIM:			2.50%					

SPEARMAN-KARBER ESTIMATES: EC50: 8.66
 VAR OF LN OF EST. : .73618D-02
 95% LOWER CONFIDENCE: 7.30
 95% UPPER CONFIDENCE: 10.28

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 2/3/97
 TEST NUMBER: 1
 DURATION: 4 D
 CHEMICAL: 2-AAF PB
 SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	
MORTALITIES:	3	5	23	40	40	40	0	0

SPEARMAN-KARBER TRIM: 7.50%

SPEARMAN-KARBER ESTIMATES: EC50: 12.06
VAR OF LN OF EST. : .50053D-02
95% LOWER CONFIDENCE: 10.47
95% UPPER CONFIDENCE: 13.89

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	37	40	40	40	0	0
MORTALITIES:	1	22	37	40	40	0	0	0
SPEARMAN-KARBER TRIM:			2.50%					

SPEARMAN-KARBER ESTIMATES: EC50: 7.16
VAR OF LN OF EST. : .31200D-02
95% LOWER CONFIDENCE: 6.41
95% UPPER CONFIDENCE: 8.01

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D

CHEMICAL: 2-AAF INH
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)		3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0	
MORTALITIES:	1	10	25	40	40	40	0	0	0
SPEARMAN-KARBER TRIM:			2.50%						

SPEARMAN-KARBER ESTIMATES: EC50: 10.74
VAR OF LN OF EST. : .45112D-02
95% LOWER CONFIDENCE: 9.39
95% UPPER CONFIDENCE: 12.28

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF AROCLOR
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)		3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0	
MORTALITIES:	3	23	40	40	40	40	0	0	0
SPEARMAN-KARBER TRIM:			7.50%						

SPEARMAN-KARBER ESTIMATES: EC50: 6.98
VAR OF LN OF EST. : .43573D-02
95% LOWER CONFIDENCE: 6.11
95% UPPER CONFIDENCE: 7.96

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 2/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF MIX
SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/L)	3.00	9.60	11.00	30.00	.00	.00	.00	.
NUMBER EXPOSED:	40	38	33	6	0	0	0	0
MORTALITIES:	2	21	28	6	0	0	0	0
SPEARMAN-KARBER TRIM:			5.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 7.48
VAR OF LN OF EST. : .51617D-02
95% LOWER CONFIDENCE: 6.48
95% UPPER CONFIDENCE: 8.64

FETAX SUMMARY SHEET

Test No. 2

Test Material	2-AAF	Investigator	Font
Source	US Army SBR	Lab	SA
CAS No.		Lot No.	
Composition/Purity		Test Start Date	
Solvent		Conc.	
		Test End Date	
		Test Units (i.e., mg/ml)	mg/L

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
pH					
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %		
	: X 100 = %	: X 100 = %
Solvent Control	: X 100 = %	: X 100 = %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀		EC ₅₀	
95% Confidence limits		95% Confidence Limits	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀)

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	: X 100 = %	: X 100 = %
2500 mg/L	: X 100 = %	: X 100 = %

FETAX MORTALITY DATA

Investigator	Font	Test Material	2-AAF
Date		Test Number	2

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
FETAX							8 0	2	
FETAX							0 1	1	
MAS-PB							1 0	1	
CP+							20 20	40	
CP-							0 0	0	
3							0 0	0	
9.6							0 0	0	
11							0 0	0	
30							0 0	0	
35							0 0	0	
50							0 0	0	
57							0 1	1	
110							5 2	7	
125							16 17	33	
150							20 20	40	
3							20 20	40	
9.6							0 0	0	
11							0 0	0	
30							1 0	1	
35							17 12	29	
50							20 20	40	
57							1 1	2	
110							↓ ↓	↓ ↓	
125							↓ ↓	↓ ↓	

150 40
 MAS-AN 1
 CP+ 40
 CP- 3

PB

BWF

FETAX MORTALITY DATA

Investigator	Test Material
Date	Test Number

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
MAS - INH							1 1	2	
AH+							18 16	34	
AH-							0 0	0	
3							0 0	0	
9.6									
11									
30									
35									
50									
57									
110									
125							1 1	2	
150							3 4	7	
3							20 20	40	
9.6							0 0	0	
11							1 0	1	
30							4 2	6	
↓							20 20	40	
3							20 20	40	
9.6							0 0	0	
11							0 1	1	
30							6 3	9	
35							15 16	31	
MAS							20 20	40	
							1 0	1	

INH

Arach

CP+

CP-

20 20 40
0 0 0

FETAX MORTALITY DATA

Investigator	Test Material
Date	Test Number

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
3							0	0	
9.6							3	2	
11							4	4	
30							15	13	
35							18	20	
50							20	30	
3							0	0	
9.6							0	0	
11							0	0	
30							0	0	
35							0	0	
50							0	0	
57							3	1	
110							17	10	
125							20	20	
150							0	0	

MIX

UNACT

FETAX MALFORMATION DATA

Investigator	Test Material	2-AAR
Date	Test Number	2

MALFORMATION	CONCENTRATION																TOTAL					
	PB					BNF					INH											
Severe	F	M	CI	3	9.6	11	30	35	50	57	110	1	MC	3	9.6	11	30	M	AP	3	9.6	
Stunted																						
Gut																						
Edema																						
Multiple																						
Cardiac																						
Abdominal																						
Facial																						
Cephalic																						
Optic																						
Tail																						
Notochord																						
Flu																						
Face																						
Eye																						
Brain																						
Hemorrhage																						
Cardiac																						
Blisters																						
Other-specify																						
No. Malformed	2	1	12	2	1	6	23	40	39	33	7		1	21	3	25	39	11	1	40	6	0
Total No.	38	39	40	40	40	40	40	40	39	53	7		39	37	40	40	39	11	38	40	6	40
Comments:																						

11

3

5

FETAX MALFORMATION DATA

Investigator

Date

Test Material

2-AAF

Test Number

2

MALFORMATION	CONCENTRATION										TOTAL			
	11	30	35	50	57	110	125	M	CP	3		9.6	11	30
Severe														
Stunted														
Gut														
Edema														
Multiple														
Cardiac														
Abdominal														
Facial														
Cephalic														
Optic														
Tail														
Notochord														
Fln														
Face														
Eye														
Brain														
Hemorrhage														
Cardiac														
Blisters														
Other-specify														
No. Malformed	36	40	40	40	40	38	33	1	15	5	26	31	9	0
Total No.	40	40	40	40	40	38	33	39	40	40	39	31	9	40
Comments:	114 (cont.) 3													

Investigator		Test Material	2-AAF
Date		Test Number	2

Investigator		Test Material	2-AAF
Date		Test Number	2

[illegible]

Comments:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: PB-MAS

DURATION: 4 D

CHEMICAL: 2-AAF

SPECIES: XENOPUS

RAW DATA:

CONCENTRATION (MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	
MORTALITIES:	0	1	6	23	40	40	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: EC50: 28.08

VAR OF LN OF EST. : .33379D-02

95% LOWER CONFIDENCE: 25.02

95% UPPER CONFIDENCE: 31.52

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: BNF-MAS

DURATION: 4 D

CHEMICAL: 2-AAF

SPECIES: XENOPUS

RAW DATA:

CONCENTRATION (MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	39	40	40	0	0	
MORTALITIES:	3	25	39	23	40	40	0	0

SPEARMAN-KARBER TRIM: 7.50%

SPEARMAN-KARBER ESTIMATES: EC50: 6.72
VAR OF LN OF EST. : .42059D-02
95% LOWER CONFIDENCE: 5.90
95% UPPER CONFIDENCE: 7.65

BNF

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE:
TEST NUMBER: INH-MAS
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	
MORTALITIES:	0	11	36	40	40	0	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: EC50: 9.10
VAR OF LN OF EST. : .28339D-02
95% LOWER CONFIDENCE: 8.18
95% UPPER CONFIDENCE: 10.12

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE:
TEST NUMBER: ARO-MAS
DURATION: 4 D

CHEMICAL: 2-AAF
SPECIES: XENOPUS

RAW DATA:

CONCENTRATION (MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	39	31	40	40	40	0	0
MORTALITIES:	5	26	31	40	40	40	0	0
SPEARMAN-KARBER TRIM:			12.50%					

SPEARMAN-KARBER ESTIMATES: EC50: 6.38
VAR OF LN OF EST. : .57942D-02
95% LOWER CONFIDENCE: 5.48
95% UPPER CONFIDENCE: 7.43

ARO

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: PB/INH/BNF-MAS

DURATION: 4 D

CHEMICAL: 2-AAF

SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)		3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	35	32	40	40	40	0	0	
MORTALITIES:	0	20	32	40	40	40	0	0	0
SPEARMAN-KARBER TRIM:				.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 7.09

VAR OF LN OF EST. : .29530D-02

95% LOWER CONFIDENCE: 6.36

95% UPPER CONFIDENCE: 7.90

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: UNACT

DURATION: 4 D

CHEMICAL: 2-AAF

SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)		3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0	
MORTALITIES:	1	5	20	32	40	40	0	0	0

SPEARMAN-KARBER TRIM: 2.50%

SPEARMAN-KARBER ESTIMATES:	EC50:	14.17
VAR OF LN OF EST. :	.52331D-02	
95% LOWER CONFIDENCE:		12.26
95% UPPER CONFIDENCE:		16.38

UNACT

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.

TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.

ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: PB-MAS

DURATION: 4 D

CHEMICAL: 2-AAF

SPECIES: XENOPUS

RAW DATA:

CONCENTRATION (MG/L)	35.00	50.00	57.00	110.00	125.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	1	7	33	40	0	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: LC50: 78.68

VAR OF LN OF EST. : .11536D-02

95% LOWER CONFIDENCE: 73.51

95% UPPER CONFIDENCE: 84.21

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: BNF-MAS

DURATION: 4 D

CHEMICAL: 2-AAF

SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)		9.60	11.00	30.00	35.00	125.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0	
MORTALITIES:	0	1	29	40	40	0	0	0	0
SPEARMAN-KARBER TRIM:				.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 21.00

VAR OF LN OF EST. : .18672D-02

95% LOWER CONFIDENCE: 19.26

95% UPPER CONFIDENCE: 22.89

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: INH-MAS

DURATION: 4 D

CHEMICAL: 2-AAF

SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)		57.00	110.00	125.00	150.00	125.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0	
MORTALITIES:	0	2	7	40	40	0	0	0	0

SPEARMAN-KARBER TRIM:

.00%

SPEARMAN-KARBER ESTIMATES: LC50: *INH.MAS* 130.67
VAR OF LN OF EST. : .26987D-03
95% LOWER CONFIDENCE: 126.45
95% UPPER CONFIDENCE: 135.04

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: ARO-MAS

DURATION: 4 D

CHEMICAL: 2-AAF

SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	125.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	1	6	40	40	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 21.96

VAR OF LN OF EST. : .12918D-02

95% LOWER CONFIDENCE: 20.43

95% UPPER CONFIDENCE: 23.59

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: PB/INH/BNF-MAS

DURATION: 4 D

CHEMICAL: 2-AAF

SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)	3.00	9.60	11.00	30.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	5	8	28	38	40	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES:	LC50:	18.01	<i>MIX-MAS</i>
VAR OF LN OF EST. :	.42881D-02		
95% LOWER CONFIDENCE:		15.80	
95% UPPER CONFIDENCE:		20.53	

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE:

TEST NUMBER: UNACT

DURATION: 4 D

CHEMICAL: 2-AAF

SPECIES: XENOPUS

RAW DATA:

CONCENTRATION(MG/L)	50.00	57.00	110.00	125.00	35.00	50.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	
MORTALITIES:	0	4	22	40	38	40	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: LC50: 90.83

VAR OF LN OF EST. : .13035D-02

95% LOWER CONFIDENCE: 84.51

95% UPPER CONFIDENCE: 97.64

FETAX SUMMARY SHEET

Test No. 1

Test Material	TCE	Investigator	Fant
Source	Sigma / US Army	Lab	SA
CAS No.		Lot No.	1
Composition/Purity		Test Start Date	
Solvent		Conc.	
		Test End Date	
		Test Units (i.e., mg/ml)	mg/L

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number		
X 100 = %		
Solvent Control		
Control Length mm		
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL		412.01	
LC ₅₀	278.13/190.35/390.13/412.10/181.29	EC ₅₀ 40.17/11.90/39.70/18.02/7.47/40.86	
95% Confidence limits	370.44-458.25/158.81-206.87	95% Confidence Limits 34.64-46.60/8.49-16.64/34.04-46.31/35.39-42.17	
	339.28-421.43/167.30-216.58/341.67-437.75	14.56-12.32/6.45-8.65/	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀)

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L		
2500 mg/L		

FETAX MORTALITY DATA

Investigator	Fort	Test Material	TCE
Date		Test Number	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
FETAX							0 0	0	
FETAX							2 1	3	
Aviclor-MAS							1 0	1	
Aviclor MAS + CP							20 20	40	
CP-							0 0	0	
5							0 0	0	
16							0 0	0	
50							0 0	0	
85							0 0	0	
97							0 0	0	
152							0 0	0	
490							0 0	0	
600							12 9	21	
INH-MAS							20 20	40	
INH + AHT							0 0	0	
AHT-							20 20	40	
5							5 4	9	
16							0 0	0	
50							0 0	0	
85							0 0	0	
97							0 6	0	
152							3 2	5	
490							10 6	16	
600							20 20	40	
							20 20	40	

Investigator	Port	Test Material	TCE						
Date		Test Number	1						
Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
Mixed NAS							0 10	0	
CP+							20 20	40	
CP-							0 0	0	
AT+							40 20	40	
AT-							5 2	7	
5							0 0	0	
16							0 0	0	
50							0 0	0	
85							0 0	0	
97							1 0	1	
152							3 2	5	
490							10 8	18	
600							20 20	40	
							20 20	40	
5							0 0	0	
16							↓ ↓ ↓	↓	
50							↓ ↓ ↓	↓	
85							↓ ↓ ↓	↓	
97							↓ ↓ ↓	↓	
152							0 0	0	
490							6 7	13	
600							20 20	40	

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
Mixed NAS							0	0	
CP+							20	20	40
CP-							0	0	
AP+							40	20	40
AP-							5	2	7
5							0	0	
16							0	0	
50							0	0	
85							0	0	
97							1	0	1
152							3	2	5
490							10	8	18
600							20	20	40
							20	20	40
5							0	0	
16							1	1	
50							1	1	
85							2	2	
97							2	2	
152							0	0	
490							6	7	13
600							20	20	40

Investigator	Date	Test Material	Test Number	Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
Fort				B-NF - MAS									
				CP+							0	0	
				CP-							20	20	40
				5							0	0	0
				16							0	0	0
				50							0	0	0
				85							0	0	0
				97							0	0	0
				152							0	0	0
				490							1	0	1
				600							8	10	18
				PB - MAS							20	20	40
				CP+							0	0	0
				CP-							20	20	40
				5							0	1	1
				16							0	0	0
				50							0	0	0
				84							0	0	0
				97							0	0	0
				152							0	0	0
				490							0	0	0
				600							10	6	16
											20	20	40

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
B-NF - MAS									
CP+							0 0	0	
CP-							20 20	40	
5							0 20	0 0	
16							0 0	0 0	
50							0 0	0 0	
85							0 0	0 0	
97							0 0	0 0	
152							0 0	0 0	
490							1 0	1	
600							8 10	18	
PB - MAS							20 20	40	
CP+							0 0	0 0	
CP-							20 20	40	
5							0 1	1	
16							0 0	0 0	
50							0 0	0 0	
84							0 0	0 0	
97							0 0	0 0	
152							0 0	0 0	
490							0 0	0 0	
600							10 6	16	
							20 20	40	

FETAX MALFORMATION DATA

Investigator	Font	Test Material	TCE
Date		Test Number	1

MALFORMATION		MIX CONCENTRATION																	TOTAL				
Severe	5	16	50	85	97	152	490	M	CR	AH	5	16	50	85	97	152	5	16	50	85	97	152	140
Stunted																							
Gut																							
Edema																							
Multiple																							
Cardiac																							
Abdominal																							
Facial																							
Cephalic																							
Optic																							
Tail																							
Notochord																							
Fln																							
Face																							
Eye																							
Brain																							
Hemorrhage																							
Cardiac																							
Blisters																							
Other-specify																							
No. Malformed	3	15	40	40	40	40	27	0	0	33	10	39	40	39	35	22	0	1	21	40	40	40	27
Total No.	40	40	40	40	40	40	24	40	40	33	40	40	40	39	35	22	40	40	40	40	40	40	27
Comments:																							

Comments:

Investigator	Fort	Test Material	TCE
Date		Test Number	1

MALFORMATION	Agacha												CONCENTRATION												B2F	152	490	85	TOTAL
	F	F	M	CR	5	16	50	85	97	152	490	M	MAH	5	16	50	85	97	152	490	OP	5	16	50					
Severe																													
Stunted																													
Gut																													
Edema																													
Multiple																													
Cardiac																													
Abdominal																													
Facial																													
Cephalic																													
Optic																													
Tail																													
Notochord																													
Fin																													
Face																													
Eye																													
Brain																													
Hemorrhage																													
Cardiac																													
Blisters																													
Other-specify																													
No. Malformed	0	0	0	0	0	1	21	40	40	19	0	31	10	23	40	40	40	35	27	0	0	0	2	21					
Total No.	40	37	39	40	40	40	40	40	40	40	19	40	31	40	40	40	40	35	24	40	40	40	40	40					
Comments:																													

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 4/3/97
 TEST NUMBER: 1
 DURATION: 4 D
 CHEMICAL: TCE AROCLOR
 SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/L)	152.00	490.00	600.00	.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	0	0	0	0	
MORTALITIES:	0	21	40	0	0	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 378.13
 VAR OF LN OF EST. : .29384D-02
 95% LOWER CONFIDENCE: 339.28
 95% UPPER CONFIDENCE: 421.43

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 4/3/98
 TEST NUMBER: 1
 DURATION: 4 D
 CHEMICAL: TCE INH
 SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/L)	85.00	97.00	152.00	490.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	
MORTALITIES:	0	5	16	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 190.35
VAR OF LN OF EST. : .41661D-02
95% LOWER CONFIDENCE: 167.30
95% UPPER CONFIDENCE: 216.58

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE B-NF
SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/L)	97.00	152.00	490.00	600.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	1	18	40	0	0	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: LC50: 390.13
VAR OF LN OF EST. : .33159D-02
95% LOWER CONFIDENCE: 347.69
95% UPPER CONFIDENCE: 437.75

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D

CHEMICAL: TCE PB
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	152.00	490.00	600.00	600.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	16	40	40	0	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 412.01
VAR OF LN OF EST. : .28279D-02
95% LOWER CONFIDENCE: 370.44
95% UPPER CONFIDENCE: 458.25

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	50.00	85.00	97.00	152.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	1	5	18	40	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 181.29
VAR OF LN OF EST. : .43559D-02
95% LOWER CONFIDENCE: 158.87
95% UPPER CONFIDENCE: 206.87

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 4/3/97
 TEST NUMBER: 1
 DURATION: 4 D
 CHEMICAL: TCE AROCLOR
 SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	15.00	50.00	85.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	
MORTALITIES:	0	1	21	40	40	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 40.17
 VAR OF LN OF EST. : .54973D-02
 95% LOWER CONFIDENCE: 34.64
 95% UPPER CONFIDENCE: 46.60

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 4/3/97
 TEST NUMBER: 1
 DURATION: 4 D
 CHEMICAL: TCE INH
 SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	16.00	50.00	85.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	
MORTALITIES:	10	23	40	40	40	0	0	0

SPEARMAN-KARBER TRIM: 25.00%

SPEARMAN-KARBER ESTIMATES: LC50: 11.90
VAR OF LN OF EST. : .28590D-01
95% LOWER CONFIDENCE: 8.49
95% UPPER CONFIDENCE: 16.69

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE B-NF
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	16.00	50.00	85.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	
MORTALITIES:	0	2	21	40	40	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 39.70
VAR OF LN OF EST. : .59211D-02
95% LOWER CONFIDENCE: 34.04
95% UPPER CONFIDENCE: 46.31

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D

CHEMICAL: TCE
SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/L)		5.00	16.00	50.00	85.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0	
MORTALITIES:	3	15	40	40	40	0	0	0	0
SPEARMAN-KARBER TRIM:				7.50%					

SPEARMAN-KARBER ESTIMATES: EC50: 18.02
VAR OF LN OF EST. : .11409D-01
95% LOWER CONFIDENCE: 14.56
95% UPPER CONFIDENCE: 22.32

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 39 10
CHEMICAL: TCE MIX
SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/L)		5.00	16.00	50.00	85.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0	
MORTALITIES:	10	39	40	40	40	0	0	0	0
SPEARMAN-KARBER TRIM:				25.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 7.47
VAR OF LN OF EST. : .53655D-02
95% LOWER CONFIDENCE: 6.45
95% UPPER CONFIDENCE: 8.65

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 4/3/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	16.00	50.00	85.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	
MORTALITIES:	0	1	21	40	0	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 40.86
VAR OF LN OF EST. : .51548D-02
95% LOWER CONFIDENCE: 35.39
95% UPPER CONFIDENCE: 47.17

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 4/3
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	152.00	490.00	600.00	85.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	
MORTALITIES:	0	16	40	40	0	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 412.01
VAR OF LN OF EST. : .28279D-02
95% LOWER CONFIDENCE: 370.44
95% UPPER CONFIDENCE: 458.25

FETAX SUMMARY SHEET

Test No. 2

Test Material	TCE	Investigator	Font
Source	Sigma / US Army SP212	Lab	STA
CAS No.	Lot No.	Test Start Date	
Composition/Purity		Test End Date	
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/L

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Solvent Control	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	383.49/175.27/388.93/410.75/167.11	EC ₅₀ 31.29/11.45/40.22/19.46/7.60	36.23
95% Confidence limits 341.64-430.47/153.43-200.22 95% Confidence Limits 32.04-43.40/8.65-15.16/34.77-47.34			
344.62-438.94/367.06-459.63/147.80-193.49 16.41-23.07/6.35-9.10/30.84-			

384.46-
472.92

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀)

42.56

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

Investigator	Font	Test Material	TCLP
Date		Test Number	3

[illegible]

Investigator	Font	Test Material	TCE
Date		Test Number	2

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
Mixed MAS							0	0	
CP+							20	40	
CP-							3	4	7
AA+							18	17	35
AA-							5	2	7
5							0	0	0
16							0	0	0
50							0	0	0
85							1	0	1
97							5	4	9
153							13	8	20
490							20	26	40
600							20	20	40
5							0	0	0
16							0	0	0
50							0	0	0
85							0	0	0
97							0	0	0
153							0	0	0
490							0	6	0
600							8	6	14
							20	20	40

Investigator	Fert	Test Material	TCE
Date		Test Number	2

[illegible]

Investigator	Font	Test Material	TCE
Date		Test Number	2

[illegible]

Investigator	Part	Test Material	TCE
Date		Test Number	2

MALFORMATION	CONCENTRATION																								TOTAL
	PB												no MAS												
	M	CP-	5	16	50	85	97	153	490	M	CP- W-	5	16	50	85	97	153	5	16	50	85	97	153	490	
Severe																									
Stunted																									
Gut																									
Edema																									
Multiple																									
Cardiac																									
Abdominal																									
Facial																									
Cephalic																									
Optic																									
Tail																									
Notochord																									
Fln																									
Face																									
Eye																									
Brain																									
Hemorrhage																									
Cardiac																									
Blisters																									
Other-specify																									
No. Malformed	0	0	0	13	40	40	40	39	25	0	0	32	11	36	40	39	20	0	3	24	40	40	40	26	
Total No.	40	36	40	40	40	40	40	39	25	40	33	40	40	40	39	31	20	40	3	24	40	40	40	24	
Comments:																									

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 5/5/97
 TEST NUMBER: 2
 DURATION: 4 D
 CHEMICAL: TCE
 SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	97.00	152.00	490.00	600.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	2	17	40	0	0	0	0
SPEARMAN-KARBER TRIM:	.00%							

SPEARMAN-KARBER ESTIMATES: LC50: 388.93
 VAR OF LN OF EST. : .36583D-02
 95% LOWER CONFIDENCE: 344.62
 95% UPPER CONFIDENCE: 438.94

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 5/5/97
 TEST NUMBER: 2
 DURATION: 4 D
 CHEMICAL: TCE PB
 SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	97.00	152.00	490.00	600.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	1	15	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 410.75
VAR OF LN OF EST. : .31613D-02
95% LOWER CONFIDENCE: 367.06
95% UPPER CONFIDENCE: 459.63

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 5/5/97
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE MIX
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	50.00	85.00	97.00	152.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	
MORTALITIES:	0	1	9	20	40	0	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: LC50: 169.11
VAR OF LN OF EST. : .45342D-02
95% LOWER CONFIDENCE: 147.80
95% UPPER CONFIDENCE: 193.49

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 5/5/97
TEST NUMBER: 2
DURATION: 4 D

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 5/5/97
 TEST NUMBER: 2
 DURATION: 4 D
 CHEMICAL: TCE AROCLOR
 SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	97.00	152.00	490.00	600.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	1	19	40	0	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 383.49
 VAR OF LN OF EST. : .33380D-02
 95% LOWER CONFIDENCE: 341.64
 95% UPPER CONFIDENCE: 430.47

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 5/7/97
 TEST NUMBER: 2
 DURATION: 4 D
 CHEMICAL: FETAX
 SPECIES: TCE

RAW DATA:

CONCENTRATION(MG/L)	85.00	97.00	152.00	490.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	0
MORTALITIES:	0	8	19	40	0	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES:	LC50:	175.27
VAR OF LN OF EST. :	.44267D-02	
95% LOWER CONFIDENCE:		153.43
95% UPPER CONFIDENCE:		200.22

CHEMICAL: TCE
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	152.00	490.00	600.00	152.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	14	40	20	40	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 426.40
VAR OF LN OF EST. : .26806D-02
95% LOWER CONFIDENCE: 384.46
95% UPPER CONFIDENCE: 472.92

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 5/6/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: TCE INH
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	16.00	50.00	85.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	8	25	40	40	40	0	0	0
SPEARMAN-KARBER TRIM:			20.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 11.45
VAR OF LN OF EST. : .19686D-01
95% LOWER CONFIDENCE: 8.65
95% UPPER CONFIDENCE: 15.16

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 5/6/97
 TEST NUMBER: 1
 DURATION: 4 D
 CHEMICAL: TCE aN
 SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	16.00	50.00	85.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	
MORTALITIES:	0	3	19	40	40	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 40.22
 VAR OF LN OF EST. : .66460D-02
 95% LOWER CONFIDENCE: 34.17
 95% UPPER CONFIDENCE: 47.34

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 5/6
 TEST NUMBER: 2
 DURATION: 4 D
 CHEMICAL: FETAX
 SPECIES: TCE

RAW DATA:

CONCENTRATION(MG/L)	5.00	16.00	50.00	85.00	490.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	0	0	0	
MORTALITIES:	0	13	40	40	40	0	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES:	EC50:	19.46
VAR OF LN OF EST. :	.72694D-02	
95% LOWER CONFIDENCE:		16.41
95% UPPER CONFIDENCE:		23.07

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 5/6/97
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: TCE NO MAS
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	5.00	16.00	50.00	85.00	.00	.00	.00	.
NUMBER EXPOSED:	40	40	40	0	0	0	0	
MORTALITIES:	0	3	24	40	0	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 36.23
VAR OF LN OF EST. : .64825D-02
95% LOWER CONFIDENCE: 30.84
95% UPPER CONFIDENCE: 42.56

SBIR Unactivated Definitive

UNACTIVATED Cyclophosphamide			
Concentration (mg/mL)	FX (mL)	Stock (mL)	
5.5 6-AN			
2500 6-AN			
3.5	4.706	3.294	
4	4.235	3.765	
4.5	3.765	4.235	
5	3.294	4.706	
5.5	2.824	5.176	
6	2.353	5.647	
6.5	1.882	6.118	
7	1.412	6.588	
7.5	0.941	7.059	
8	0.471	7.529	
8.5	0.000	8.000	
Sum		62.118	
x 2 dishes		124.2353	
x 4 days		496.9412	

8.5 mg/mL x 500 mL
= 4,250 g

UNACTIVATED Trichlorethylene			
Concentration (mg/mL)	FX (mL)	Stock (mL)	
5.5 6-AN			
2500 6-AN			
0.035	7.533	0.467	
0.04	7.467	0.533	
0.045	7.400	0.600	
0.05	7.333	0.667	
0.055	7.267	0.733	
0.06	7.200	0.800	
0.065	7.133	0.867	
0.09	4.000	4.000	
0.35	3.333	4.667	
0.4	2.667	5.333	
0.45	2.000	6.000	
0.5	1.333	6.667	
0.55	0.667	7.333	
0.6	0.000	8.000	

Sum 46.667
x 2 dishes 93.33333
x 4 days 373.3333

0.6 mg/mL x 500 mL
= 300 mg

UNACTIVATED 2-AAF			
Concentration (mg/mL)	FX (mL)	Stock (mL)	
5.5 6-AN			
2500 6-AN			
0.02	7.300	0.700	
0.025	7.200	0.800	
0.03	7.000	0.900	
0.035	6.800	1.200	
0.04	6.700	1.300	
0.045	6.500	1.500	
0.05	6.400	1.600	
0.09	4.000	4.000	
0.1	3.200	4.800	
0.15	2.400	6.000	
0.2	1.600	8.000	
0.25	0.800		

Sum 33.280
x 2 dishes 66.56
x 4 days 266.24

0.25 mg/mL x 500 mL

5.000 mg

UNACTIVATED 2-AAF			
Concentration (mg/mL)	FX (mL)	Stock (mL)	
5.5 6-AN			
2500 6-AN			
0.045	6.560	1.440	
0.05	6.400	1.600	
0.055	6.240	1.760	
0.06	6.080	1.920	
0.065	5.920	2.080	
0.07	5.760	2.240	
0.075	5.600	2.400	
0.095	4.960	3.040	
0.1	4.800	3.200	
0.15	3.200	4.800	
0.2	1.600	6.400	
0.25	0.000	8.000	

Sum 38.880
x 2 dishes 77.76
x 4 days 311.04

0.25 mg/mL x 0.5 L

0.000 mg

Unactivated Definitive Range test 2-AAF

~~2-AAF~~ Mechanism of Action-I
9-23-96

UNACTIVATED 2-AAF DEFINITIVE					
Concentration (mg/L)	FX (mL)	Stock (mL)	MAS (mL)	Generator (mL)	Enzyme (mL) Other
FX Controls					
8					
5.5 6-AN Control					
2500 6-AN Control					
0.02	6.933	1.067			
0.025	6.667	1.333			
0.03	6.400	1.600			
0.035	6.133	1.867			
0.04	5.867	2.133			
0.045	5.600	2.400			
0.05	5.333	2.667			
0.09	3.200	4.800			
0.095	2.933	5.067			
0.1	2.667	5.333			
0.125	1.333	6.667			
0.15	0.000	8.000			

Thalidomide Mechanism of Action

THIOACETAMIDE UNACTIVATED RANGE						
Concentration (mg/mL)	FX (mL)	Stock A (mL)	Stock B (mL)	MAS (mL)	Generator (mL)	Enzyme (mL) Other
FX Controls						
5.5 6-AN Control	8					
2500 6-AN Control						
0.005	7.920		0.080			
0.01	7.840		0.160			
0.05	7.200		0.800			
0.1	6.400		1.600			
0.5	0.000		8.000			
1	7.680	0.320				
2.5	7.200	0.800				
5	6.400	1.600				
10	4.800	3.200				
25	0.000	8.000				

²⁵
 Stock solution A = 25 mg/mL
 Total volume of stock A required to finish one (1) test = 111.36 mL
^{0.5}
 Stock solution B = 0.5 mg/mL
 Total volume of stock B required to finish one (1) test = 85.12

$$25 \text{ mg/mL} \times 200 \text{ mL} = 5000 \text{ mg}$$

FETAX SUMMARY SHEET

Test Material <u>Cyphosphamide</u>		Test No. <u>1</u>
Source <u>Sigma Chemical</u>	Investigator <u>Fort</u>	
CAS No.	Lab <u>SA</u>	
Lot No.	Test Start Date	
Composition/Purity	Test End Date	
Solvent	Conc.	Test Units (i.e., mg/ml) <u>mg/mL</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>— pH —</u>					
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %		
	<u>0</u> : <u>80</u> X 100 = <u>0</u> %	<u>0</u> : <u>80</u> X 100 = <u>0</u> %
Solvent Control	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

no MA, β -NF, INH, Arocl, PB, mix

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀ > 4.0, 3.09, > 4.0, 1.50, 1.19, 1.34	EC ₅₀ > 4.0, 1.81, > 4.0, 0.59, 0.61, 0.40		
95% Confidence limits ND, 3.0-3.17, ND 1.02-1.58, 1.11-1.81, 1.26-1.43	95% Confidence Limits ND, 1.72-1.91, ND 0.51-0.68, 0.53-0.70, 0.34-0.47		
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀) ND, 1.71, ND, 2.54, 2.79, 3.35			

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
2500 mg/L	_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %

FETAX MORTALITY DATA

Investigator	Font	Test Material	Cylophosphamide
Date	12/4/96	Test Number	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
FETAX							0	0	0
FETAX							0	0	0
PB-MAS							0	0	0
B-NF-MAS							0	0	0
INT-MAS							0	0	0
Agar-MAS							0	0	0
MIX-MAS							0	0	0
0.1							0	0	0
0.5							1	1	1
0.75							1	1	1
1							1	1	1
1.25							1	1	1
1.5							1	1	1
2							1	1	1
2.5							1	1	1
3							1	1	1
3.5							1	1	1
4							3	3	3
0.1							0	0	0
0.5							0	0	0
0.75							0	0	0
1							0	0	0
1.25							0	0	0

no
MAS

B-NF

1.5
2
2.5
3
3.5
4
0
0
2
3
20
20
0
0
1
5
20
20
0
0
3
8
40
100

FETAX MORTALITY DATA

Investigator	Font	Test Material	CPA
Date	12/7/96	Test Number	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
0.1							0	0	
0.5							1	1	
0.75							1	1	
1							1	1	
1.25							1	1	
1.5							1	1	
2.0							1	1	
2.5							3	3	
3							5	5	
3.5							5	5	
4							9	9	
0.1							0	0	
0.5							0	0	
0.75							0	0	
1							2	2	
1.25							3	3	
1.5							12	12	
2.0							18	18	
2.5							20	20	
3							20	20	
3.5							20	20	
4							20	20	

FMH

Ar. J. J.

FETAX MORTALITY DATA

Investigator	For +	Test Material	CPA
Date	12/4/90	Test Number	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
0.1							0	0	
0.5							0	0	
0.75							2	0	
1							5	3	
1.25							8	3	
1.5							12	10	
2							20	22	
2.5							20	40	
3							↓	↓	
3.5							↓	↓	
4							↓	↓	
0.1							0	0	
0.5							0	0	
0.75							0	0	
1							0	1	
1.25							4	3	
1.5							8	5	
2							10	14	
2.5							20	20	
3							20	40	
3.5							↓	↓	
4							↓	↓	

CP - Aro MAS
 CP - INT MAS
 CP - BNF MAS
 CP - PB MAS
 CP - MIX MAS

0 0 0 0 0 0 0 0 0 0
 0 0 0 0 0 0 0 0 0 0
 2 1 1 0 1 0 1 0 1 1

MIX

PB

FETAX MALFORMATION DATA

Investigator	For J	Test Material	CPA
Date	12/4/96	Test Number	

MALFORMATION	MAS										CONCENTRATION										NO MAS					TOTAL
	F	F	DF	MA	PA	A	M	DF	MA	PA	A	M	0.1	0.5	0.15	1	1.25	1.5	2	2.5	3	3.5	4			
Severe																										
Stunted																										
Gut																										
Edema																										
Multiple																										
Cardiac																										
Abdominal																										
Facial																										
Cephalic																										
Optic																										
Tail																										
Notochord																										
Fin																										
Face																										
Eye																										
Brain																										
Hemorrhage																										
Cardiac																										
Blisters																										
Other-specify																										
No. Malformed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	6	10			
Total No.	40	40	40	40	40	40	40	40	40	40	40	37	40	39	40	—	—	—	—	—	→	39	35			
Comments:																										

Comments:

FETAX MALFORMATION DATA

Investigator	For J	Test Material	CRA
Date		Test Number	1

MALFORMATION	CONCENTRATION																TOTAL
	0.1	0.15	0.25	0.35	0.4	0.5	0.75	1	1.25	1.5	2	2.5	3	3.5	4		
Severe																	
Stunted																	
Gut																	
Edema																	
Multiple																	
Cardiac																	
Abdominal																	
Facial																	
Cephalic																	
Optic																	
Tail																	
Notochord																	
Fin																	
Face																	
Eye																	
Brain																	
Hemorrhage																	
Cardiac																	
Blisters																	
Other-specify																	
No. Malformed	0	0	0	-	-	0	0	0	0	0	1	5	6	6	10		
Total No.	40	40	40	40	40	40	40	40	40	40	40	37	32	32	26		

Comments:

FETAX MALFORMATION DATA

Investigator	Font	Test Material	CPA
Date		Test Number	1

MALFORMATION	CONCENTRATION										TOTAL
	0.1	0.5	1	1.25	1.5	2	3	2.5	2	1.5	
Severe	0.1	0.5	1	1.25	1.5	2	3	2.5	2	1.5	2
Stunted											
Gut											
Edema											
Multiple											
Cardiac											
Abdominal											
Facial											
Cephalic											
Optic											
Tail											
Notochord											
Fin											
Face											
Eye											
Brain											
Hemorrhage											
Cardiac											
Blisters											
Other-specify											
No. Malformed	0	8	23	35	33	9	5	5	5	27	16
Total No.	40	40	40	37	33	9	5	5	5	27	16

Comments:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/4/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CPA B-NF
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	2.00	2.50	3.00	3.50	4.00	.00	.00
NUMBER EXPOSED:	40	40	40	40	0	0	0
MORTALITIES:	0	3	8	40	40	0	0
SPEARMAN-KARBER TRIM:	.00%						

SPEARMAN-KARBER ESTIMATES: LC50: 3.09
VAR OF LN OF EST. : .18450D-03
95% LOWER CONFIDENCE: 3.00
95% UPPER CONFIDENCE: 3.17

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

VOID

DATE: 12/4
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CP INH
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	2.00	2.50	3.00	3.50	4.00	.00	.00
NUMBER EXPOSED:	40	40	40	40	0	0	0
MORTALITIES:	0	3	8	27	38	0	0

SPEARMAN-KARBER TRIM: 5.00%

SPEARMAN-KARBER ESTIMATES: LC50: 3.28
VAR OF LN OF EST. : .33661D-03
95% LOWER CONFIDENCE: 3.16
95% UPPER CONFIDENCE: 3.40

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/4/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CPA AROCLOR
SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/ML)		.75	1.00	1.25	1.50	2.00	2.50	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0	
MORTALITIES:	0	3	7	21	35	40	0	0	0
SPEARMAN-KARBER TRIM:				.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 1.50
VAR OF LN OF EST. : .78417D-03
95% LOWER CONFIDENCE: 1.42
95% UPPER CONFIDENCE: 1.58

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/4
TEST NUMBER: 1
DURATION: 4 D

CHEMICAL: CPA - PB
SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/ML)		.75	1.00	1.25	1.50	2.00	2.50	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0	
MORTALITIES:	0	1	7	13	24	40	0	0	0
SPEARMAN-KARBER TRIM:				.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 1.70
VAR OF LN OF EST. : .88239D-03
95% LOWER CONFIDENCE: 1.61
95% UPPER CONFIDENCE: 1.81

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/4
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CPA
SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/L (M))		.50	.75	1.00	1.25	1.50	2.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0	
MORTALITIES:	0	2	8	11	22	40	0	0	0
SPEARMAN-KARBER TRIM:				.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 1.34
VAR OF LN OF EST. : .95015D-03
95% LOWER CONFIDENCE: 1.26
95% UPPER CONFIDENCE: 1.43

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 12/4
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CPA - BNF
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	1.00	1.25	1.50	2.00	2.50	2.00	.00	.
NUMBER EXPOSED:	40	40	40	40	37	40	0	0
MORTALITIES:	0	1	10	23	37	40	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 1.81
VAR OF LN OF EST. : .68247D-03
95% LOWER CONFIDENCE: 1.72
95% UPPER CONFIDENCE: 1.91

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:
HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/4
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CPA - INH
SPECIES: FETAX

VOID

RAW DATA:

CONCENTRATION(MG/ML)	1.50	2.00	2.50	3.00	3.50	2.00	.00	.
NUMBER EXPOSED:	40	40	37	32	13	40	0	0
MORTALITIES:	0	1	5	19	13	40	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 2.83
VAR OF LN OF EST. : .38293D-03
95% LOWER CONFIDENCE: 2.73
95% UPPER CONFIDENCE: 2.95

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/4
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CPA - AROCLOR
SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/ML)		.10	.50	.75	1.00	1.25	2.00	.00	.
NUMBER EXPOSED:	40	40	40	37	35	40	0	0	
MORTALITIES:	0	8	23	35	35	40	0	0	0
SPEARMAN-KARBER TRIM:				.00%					

SPEARMAN-KARBER ESTIMATES: EC50: .59
VAR OF LN OF EST. : .48838D-02
95% LOWER CONFIDENCE: .51
95% UPPER CONFIDENCE: .68

FETAX SUMMARY SHEET

Test No. 2

Test Material <u>Cyclophosphamide</u>	Investigator <u>Font</u>
Source <u>Sigma chemical</u>	Lab <u>S+A</u>
CAS No. _____ Lot No. _____	Test Start Date _____
Composition/Purity _____	Test End Date _____
Solvent _____ Conc. _____	Test Units (i.e., mg/ml) <u>mg/mL</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u> </u> pH <u> </u>					
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %		
	<u>2</u> : <u>80</u> X 100 = <u>2.5</u> %	<u>2</u> : <u>78</u> X 100 = <u> </u> %
Solvent Control <u>MAS</u>	<u>0</u> : <u>40</u> X 100 = <u>0</u> %	<u>3</u> : <u>40</u> X 100 = <u>7.5</u> %
Control Length <u> </u> mm	Solvent Control Length <u> </u> mm	
Minimum Concentration to Inhibit Growth (MCIG)		

inactive to B-NF/INH/Arach/PB/mix

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	<u>>4.00/3.13/4.00/1.85/1.59/1.69</u>	EC ₅₀	<u>>4.00/1.86/74.00/0.71/0.92/0.72</u>
95% Confidence limits	<u>ND/3.00-3.28/ND/1.74-1.98</u> <u>1.51-1.67/1.58-1.81</u>	95% Confidence Limits	<u>1.15-1.97/ND/0.62-0.80</u> <u>0.87-0.97/0.63-0.81</u>

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) ND/1.68/ND/2.61/1.73 2.35

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
2500 mg/L	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %

Investigator	Font	Test Material	CP (B-NF - MAS)
Date	12/30/96	Test Number	2
Concentration			

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
FETAX									
FETAX									
MAS									
CP+							0 1	1	
CP-							20 20	40	
							1 1	2	
0.1									
0.5							0 1	1	
0.75							0 0	0	
1							0 0	0	
1.25							0 0	0	
1.5							0 0	0	
2							0 0	0	
2.5							0 2	2	
3							2 4	6	
3.5							7 7	14	
4							10 11	21	
							20 20	40	

Investigator	Font		Test Material	CP (BNF - MAS)
Date	12	30/96	Test Number	2

MALFORMATION	CONCENTRATION													TOTAL
	F	M	OP-	0.1	0.5	0.75	1	1.25	1.5	2	2.5	3	3.5	
Severe		1	38											
Stunted														
Gut														
Edema														
Multiple														
Cardiac														
Abdominal														
Facial														
Cephalic														
Optic														
Tail														
Notochord														
Fln														
Face														
Eye														
Brain														
Hemorrhage														
Cardiac														
Blisters														
Other-specify														
No. Malformed		1	38		0	0	0	3	10	20	30	26	19	
Total No.		39	38		40	40	40	40	40	38	34	26	19	
Comments:														

Investigator	Fort	Test Material	Op (INH-MAS)
Date	12/20/96	Test Number	2
Concentration			

[illegible]

FETAX MALFORMATION DATA

Investigator	For +	Test Material	CP (INIT-MAS)
Date	12/31/96	Test Number	2

MALFORMATION	CONCENTRATION												TOTAL
	M	CP-	0.1	0.5	0.75	1	1.25	1.5	2	2.5	3	3.5	4
Severe													
Stunted													
Gut													
Edema													
Multiple													
Cardiac													
Abdominal													
Facial													
Cephalic													
Optic													
Tail													
Notochord													
Fin													
Face													
Eye													
Brain													
Hemorrhage													
Cardiac													
Blisters													
Other-specify													
No. Malformed	0	40	0						→	3	5	5	10
Total No.	40	40	40						→	40	40	35	75

Comments:

Investigator	For	Test Material	Test Number	Test Date	Test Time	Test Location	Test Method	Test Result	Test Comment
Investigator	For	Test Material	Test Number	Test Date	Test Time	Test Location	Test Method	Test Result	Test Comment
Date	12/30/16	CP (Aroclor MAS)	2						
Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
MAS							1	0	
CP+							20	20	
CP-							1	2	
0.1							0	0	
0.5							0	0	
0.75							0	0	
1							0	0	
1.25							1	4	
1.5							8	6	
2							12	11	
2.5							15	12	
3							20	20	
3.5							20	20	
4							20	20	

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
MAS							1 0	1	
UP+							20 20	40	
UP-							1 2	3	
0.1							0 0	0	
0.5							0 0	0	
0.75							0 0	0	
1							0 0	0	
1.25							1 4	5	
1.5							8 6	14	
2							12 11	23	
2.5							15 12	27	
3							20 20	40	
3.5							20 20	40	
4							20 20	40	

FETAX MALFORMATION DATA

Investigator	Font	Test Material	CP (Aroclor - MAS)
Date	12/31/96	Test Number	2

MALFORMATION	CONCENTRATION											TOTAL
	M	CP	0.1	0.5	0.75	1	1.25	1.5	2	2.5		
Severe	1	25										
Stunted												
Gut												
Edema												
Multiple												
Cardiac												
Abdominal												
Facial												
Cephalic												
Optic												
Tail												
Notochord												
Fin												
Face												
Eye												
Brain												
Hemorrhage												
Cardiac												
Bladders												
Other-specify												
No. Malformed	1	25	0	5	18	28	35	26	17	13		
Total No.	39	37	40	40	40	40	35	26	17	13		

Comments:

FETAX MORTALITY DATA

Investigator	Farr		Test Material	CP	PB-MAS
Date	12/30/96		Test Number	2	

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
MAS							0	0	
CP							20	20	
CP-							0	0	
0.1							0	0	
0.5							0	0	
0.75							0	0	
1							0	1	
1.25							3	2	
1.5							8	8	
2							17	17	
2.5							20	20	
3							20	20	
3.5							20	20	
4							20	20	

FETAX MALFORMATION DATA

Investigator	Fort	Test Material	CP (PB-MAS)
Date	12/30/96	Test Number	2

MALFORMATION		CONCENTRATION										TOTAL
M	U-	0.1	0.5	0.75	1	1.25	1.5	2				
Severe	30											
Stunted												
Gut												
Edema												
Multiple												
Cardiac												
Abdominal												
Facial												
Cephalic												
Optic												
Tail												
Notochord												
Fln												
Face												
Eye												
Brain												
Hemorrhage												
Cardiac												
Blisters												
Other-specify												
No. Malformed	0	0	0	5	23	35	14	6				
Total No.	40	40	40	40	39	35	14	6				
Comments:												

Investigator	Font	Test Material	cyclophosphamide
Date	12/30/96	Test Number	2
Concentration			

[illegible]

Investigator	Font	Test Material	CP (unactivated)
Date	12/30/96	Test Number	2

[illegible]

FETAX MALFORMATION DATA

Investigator	Font	Test Material	CP (uninjected)
Date	12/30/96	Test Number	2

MALFORMATION	CONCENTRATION												TOTAL
	F	F	0.1	0.5	0.75	1	1.25	1.5	2	2.5	3	3.5	4
Severe	1	1											
Stunted													
Gut													
Edema													
Multiple													
Cardiac													
Abdominal													
Facial													
Cephalic													
Optic													
Tail													
Notochord													
Fln													
Face													
Eye													
Brain													
Hemorrhage													
Cardiac													
Blisters													
Other-specify													
No. Malformed	1	1	0	0	0	0	0	0	0	0	1	2	5
Total No.	39	39	40								39	35	

Comments:

FETAX MALFORMATION DATA

Investigator	Font	Test Material	CP (MIX)
Date	12/31/9V	Test Number	2

MALFORMATION	CONCENTRATION											TOTAL
	M	CP	0.1	0.5	0.75	1	1.25	1.5	2	2.5	3	
Severe	2	37										
Stunted												
Gut												
Edema												
Multiple												
Cardiac												
Abdominal												
Facial												
Cephalic												
Optic												
Tail												
Notochord												
Flu												
Face												
Eye												
Brain												
Hemorrhage												
Cardiac												
Blisters												
Other-specify												
No. Malformed	2	37	0	5	16	27	34	20	15	8	1	
Total No.	40	37	40	40	40	38	34	20	15	8	1	

Comments:

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 12/31/96
 TEST NUMBER: 2
 DURATION: 4 D
 CHEMICAL: CP B-NF MAS
 SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	1.50	2.00	2.50	3.00	3.50	4.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	
MORTALITIES:	0	2	6	14	21	40	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 3.13
 VAR OF LN OF EST. : .49844D-03
 95% LOWER CONFIDENCE: 3.00
 95% UPPER CONFIDENCE: 3.28

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 12/31/96
 TEST NUMBER: 2
 DURATION: 4 D
 CHEMICAL: CP
 SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	1.00	1.25	1.50	2.00	2.50	3.00	.00	.
NUMBER EXPOSED:	40	40	40	38	34	26	0	0
MORTALITIES:	0	3	10	20	30	26	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES:	EC50:	1.86
VAR OF LN OF EST. :	.88363D-03	
95% LOWER CONFIDENCE:		1.75
95% UPPER CONFIDENCE:		1.97

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 12/31/96
 TEST NUMBER: 2
 DURATION: 4 D
 CHEMICAL: CP AROCLOR-MAS
 SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/ML)	1.00	1.25	1.50	2.00	2.50	3.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	
MORTALITIES:	0	5	14	23	27	40	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: LC50: 1.85
 VAR OF LN OF EST. : .10504D-02
 95% LOWER CONFIDENCE: 1.74
 95% UPPER CONFIDENCE: 1.98

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
 TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 12/31/96
 TEST NUMBER: 2
 DURATION: 4 D
 CHEMICAL: CP AROCLOR MAS
 SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/ML)	.10	.50	.75	1.00	1.25	3.00	.00	.
NUMBER EXPOSED:	40	40	40	40	35	40	0	
MORTALITIES:	0	5	18	28	35	40	0	0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: EC50: .71
VAR OF LN OF EST. : .38610D-02
95% LOWER CONFIDENCE: .62
95% UPPER CONFIDENCE: .80

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
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LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/31/96
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: CP PB-MAS
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	.75	1.00	1.25	1.50	2.00	2.50	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	
MORTALITIES:	0	1	5	16	34	40	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: LC50: 1.59
VAR OF LN OF EST. : .69143D-03
95% LOWER CONFIDENCE: 1.51
95% UPPER CONFIDENCE: 1.67

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

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LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/31/96
TEST NUMBER: 2
DURATION: 4 D

CHEMICAL: CP PB-MAS
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)		.50	.75	1.00	1.25	.00	.00	.00	.
NUMBER EXPOSED:	40	40	39	35	0	0	0	0	
MORTALITIES:	0	5	23	35	0	0	0	0	0
SPEARMAN-KARBER TRIM:				.00%					

SPEARMAN-KARBER ESTIMATES: LC50: .92
VAR OF LN OF EST. : .73314D-03
95% LOWER CONFIDENCE: .87
95% UPPER CONFIDENCE: .97

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 12/31/96
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: CP MIX-MAS
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)		.75	1.00	1.25	1.50	2.00	2.50	3.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	40	0	
MORTALITIES:	0	2	6	20	25	32	40	0	0
SPEARMAN-KARBER TRIM:				.00%					

SPEARMAN-KARBER ESTIMATES: LC50: 1.69
VAR OF LN OF EST. : .11003D-02
95% LOWER CONFIDENCE: 1.58
95% UPPER CONFIDENCE: 1.81

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

FOR REFERENCE, CITE:

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TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;

CORRECTION 12(4):417 (1978).

DATE: 12/31/96
TEST NUMBER: 2
DURATION: 4 D
CHEMICAL: CP MIX-MAS
SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/ML)		.10	.50	.75	1.00	1.25	2.50	3.00	.
NUMBER EXPOSED:	40	40	40	38	34	40	40	0	
MORTALITIES:	0	5	16	27	34	32	40	0	0
SPEARMAN-KARBER TRIM:				.00%					

SPEARMAN-KARBER ESTIMATES: EC50: .72
VAR OF LN OF EST. : .38491D-02
95% LOWER CONFIDENCE: .63
95% UPPER CONFIDENCE: .81

⇒ C P A D u A

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Column statistics
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$\Rightarrow$ CPABNFA

SBIR CYCLOPHOSPHAMIDE
INH ACT. DEF WAS
 $\Rightarrow$ CPA INHA

$\Rightarrow$ CPAAROA

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I hereby certify that the foregoing is a true and correct copy of the original as the same appears in the records of the _____;

[illegible][illegible]

$\Rightarrow$ BNF IN HPB

[illegible]

1-13-97

$\Rightarrow$ C2AROA

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Column statistics

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I m m u n o p r e s s i v e ; I m m u n o p r e s s i v e ;

THE UNIVERSITY OF CHICAGO

I hereby certify that the foregoing is a true and correct copy of the original as the same appears from the records of the County of _____ State of _____; _____

[illegible][illegible]

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Tailly

District

Column statistics

$$\Rightarrow 0.2 \text{ uA}$$

1-13-97

$$\Rightarrow C_2INH_A$$

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1 MAS

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3 Acl+

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Distance
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Column statistics
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$$\Rightarrow C2BNFA$$

$\Rightarrow C2MIXA$

$\Rightarrow C2PBA$

$\Rightarrow$ CPBA

[illegible]

$\Rightarrow$ CAROA

[illegible]

COUMARIN MIX ACT.

$$\Rightarrow C_{mixA}$$
[illegible]

$$\Rightarrow \text{CINHA}$$

$\Rightarrow$ CBNFA

$$\Rightarrow CUA$$
[illegible]

7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000 1001 1002 1003 1004 1005 1006 1007 1008 1009 1010 1011 1012 1013 1014 1015 1016 1017 1018 1019 1020 1021 1022 1023 1024 1025 1026 1027 1028 1029 1030 1031 1032 1033 1034 1035 1036 1037 1038 1039 1040 1041 1042 104

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unavailable to
digitize

$\Rightarrow 2AFMIXA$

FETAX TEST CONCENTRATION SPREADSHEET				
COMPOUND:		COUMARIN		
EC50=	59.8			
LC50=	168.7			
EC5	5.98			
EC16	19.136			
EC50	59.8			
EC84	100.464			
EC95	113.62			
LC5	16.87			
LC16	53.984			
LC50	168.7			
LC84	283.416			
LC95	320.53			

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 11/13/96

TEST NUMBER: 1

DURATION: 4 D

CHEMICAL: CYCLOPHOSPHAMIDE

SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	5.50	6.00	6.50	7.00	7.50	8.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	
MORTALITIES:	0	2	13	23	39	40	0	0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: LC50: 6.76

VAR OF LN OF EST. : .74859D-04

95% LOWER CONFIDENCE: 6.65

95% UPPER CONFIDENCE: 6.88

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
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ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 11/13/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: CYCLOPHOSPHAMIDE
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	3.50	4.00	4.50	5.00	5.50	.00	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	0
MORTALITIES:	0	1	6	22	40	0	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: EC50: 5.83
VAR OF LN OF EST. : .11159D-03
95% LOWER CONFIDENCE: 5.71
95% UPPER CONFIDENCE: 5.95

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 11/13/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)		.05	.09	.10	.10	.13	.15	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0	
MORTALITIES:	0	1	4	8	39	40	0	0	0
SPEARMAN-KARBER TRIM:				.00%					

SPEARMAN-KARBER ESTIMATES: LC50: .11
VAR OF LN OF EST. : .16937D-03
95% LOWER CONFIDENCE: .11
95% UPPER CONFIDENCE: .11

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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HAMILTON, M.A., R.C. RUSSO, AND R.V. THURSTON, 1977.
TRIMMED SPEARMAN-KARBER METHOD FOR ESTIMATING MEDIAN
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ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 11/13/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: 2-AAF
SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)		.02	.03	.03	.04	.04	.05	.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	0	
MORTALITIES:	5	10	19	26	38	40	0	0	0
SPEARMAN-KARBER TRIM:			12.50%						

SPEARMAN-KARBER ESTIMATES: EC50: .03
VAR OF LN OF EST. : .91693D-03
95% LOWER CONFIDENCE: .03
95% UPPER CONFIDENCE: .03

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 11/13/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: TCE
SPECIES: FETAX

RAW DATA:

CONCENTRATION (MG/ML)	.35	.40	.45	.50	.55	.05	.00	.
NUMBER EXPOSED:	40	40	40	40	40	0	0	
MORTALITIES:	0	3	7	13	40	40	0	0
SPEARMAN-KARBER TRIM:			.00%					

SPEARMAN-KARBER ESTIMATES: LC50: .49
VAR OF LN OF EST. : .12753D-03
95% LOWER CONFIDENCE: .48
95% UPPER CONFIDENCE: .50

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 11/13/96
 TEST NUMBER: 1
 DURATION: 4 D
 CHEMICAL: TCE
 SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/L)	35.00	40.00	45.00	50.00	55.00	60.00	65.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	
MORTALITIES:	0	2	10	18	21	38	40	0 0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: EC50: 50.80
 VAR OF LN OF EST. : .19949D-03
 95% LOWER CONFIDENCE: 49.38
 95% UPPER CONFIDENCE: 52.25

TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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 LETHAL CONCENTRATIONS IN TOXICITY BIOASSAYS.
 ENVIRON. SCI. TECHNOL. 11(7): 714-719;
 CORRECTION 12(4):417 (1978).

DATE: 11/13/96
 TEST NUMBER: 1
 DURATION: 4 D
 CHEMICAL: COUMARIN
 SPECIES: FETAX

RAW DATA:

CONCENTRATION(MG/ML)	75.00	95.00	100.00	150.00	200.00	600.00	650.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	
MORTALITIES:	0	1	6	11	40	38	40	0 0

SPEARMAN-KARBER TRIM: .00%

SPEARMAN-KARBER ESTIMATES: LC50: 168.74
VAR OF LN OF EST. : .77755D-03
95% LOWER CONFIDENCE: 154.17
95% UPPER CONFIDENCE: 184.16

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11/13/96
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TRIMMED SPEARMAN-KARBER METHOD. MONTANA STATE UNIV

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ENVIRON. SCI. TECHNOL. 11(7): 714-719;
CORRECTION 12(4):417 (1978).

DATE: 11/13/96
TEST NUMBER: 1
DURATION: 4 D
CHEMICAL: COUMARIN
SPECIES: FETAC

RAW DATA:

CONCENTRATION(MG/L)	50.00	55.00	60.00	65.00	70.00	60.00	65.00	.
NUMBER EXPOSED:	40	40	40	40	40	40	0	
MORTALITIES:	0	11	19	29	40	38	40	0 0
SPEARMAN-KARBER TRIM:				.00%				

SPEARMAN-KARBER ESTIMATES: EC50: 59.79
VAR OF LN OF EST. : .11453D-03
95% LOWER CONFIDENCE: 58.52
95% UPPER CONFIDENCE: 61.08

FETAX TEST CONCENTRATION SPREADSHEET				
COMPOUND:	2-AAF			
EC50=	110			
LC50=	30			
EC5	11			
EC16	35.2			
EC50	110			
EC84	184.8			
EC95	209			
LC5	3			
LC16	9.6			
LC50	30			
LC84	50.4			
LC95	57			

FETAX TEST CONCENTRATION SPREADSHEET				
COMPOUND:		COUMARIN		
EC50=	59.79			
LC50=	268.74			
EC5	5.979			
EC16	19.1328			
EC50	59.79			
EC84	100.4472			
EC95	113.601			
LC5	26.874			
LC16	85.9968			
LC50	268.74			
LC84	451.4832			
LC95	510.606			

FETAX TEST CONCENTRATION SPREADSHEET				
COMPOUND:	TCE			
EC50=	490			
LC50=	51			
EC5	49			
EC16	156.8			
EC50	490			
EC84	823.2			
EC95	931			
LC5	5.1			
LC16	16.32			
LC50	51			
LC84	85.68			
LC95	96.9			

FETAX TEST CONCENTRATION SPREADSHEET				
COMPOUND:		CYCLOPHOSPHAMIDE		
EC50=	4.83			
LC50=	6.76			
EC5	0.483			
EC16	1.5456			
EC50	4.83			
EC84	8.1144			
EC95	9.177			
LC5	0.676			
LC16	2.1632			
LC50	6.76			
LC84	11.3568			
LC95	12.844			

FETAX SUMMARY SHEET

Test Material <u>Cyclophosphamide</u>		Test No. <u>1</u>
Source <u>Sigma</u>	Investigator <u>Fort</u>	
CAS No.	Lab <u>SA</u>	
Lot No.	Test Start Date	
Composition/Purity	Test End Date	
Solvent	Conc.	Test Units (i.e., mg/ml) <u>mg/mL</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u> </u> pH <u> </u>					
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number		
X 100 = %	<u>0</u> : <u>80</u> X 100 = <u>0</u> %	<u>0</u> : <u>80</u> X 100 = <u>0</u> %
Solvent Control	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	<u>6.76</u>	EC ₅₀	<u>4.83</u>
95% Confidence limits	<u>6.65 - 6.88</u>	95% Confidence Limits	<u>4.71 - 4.95</u>

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀)

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	<u>0</u> : <u>40</u> X 100 = <u>0</u> %	<u>24</u> : <u>40</u> X 100 = <u> </u> %
2500 mg/L	<u>25</u> : <u>40</u> X 100 = <u> </u> %	<u>15</u> : <u>15</u> X 100 = <u>100</u> %

Investigator	F. J. T.	Test Material	Cytoplast
Date	11/12/96	Test Number	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
FETAX							0	0	
FETAX							0	0	
6-AN 5.5							0	0	
6-AN 2500							12	13	25
3.5							0	0	
4							0	0	
4.5							0	0	
5							0	0	
5.5							0	0	
6							0	0	
6.5						2	2	0	
7						8	8	5	13
7.5						14	14	9	23
8						20	20	19	39
8.5						20	20	20	40
						20	20	20	40

Investigator	Fort	Test Material	Cyl of phosphide
Date	11/12/96	Test Number	

Comments:

FETAX SUMMARY SHEET

Test Material <u>2-AAF</u>		Test No. <u>1</u>
Source <u>Sigma</u>	Investigator <u>Fort</u>	
CAS No.	Lab <u>SA</u>	
Lot No.	Test Start Date	
Composition/Purity	Test End Date	
Solvent	Conc.	Test Units (i.e., mg/ml) <u>mg/ml</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>— pH —</u>					
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number		
X 100 = %		
	<u>0 : 80</u> X 100 = <u>0</u> %	<u>0 : 80</u> X 100 = <u>0</u> %
Solvent Control	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	<u>0.11</u>	EC ₅₀	<u>0.03</u>
95% Confidence limits	<u>0.105 - 0.115</u>	95% Confidence Limits	<u>0.025 - 0.035</u>

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀)

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	<u>0 : 40</u> X 100 = <u>0</u> %	<u>18 : 40</u> X 100 = <u>45</u> %
2500 mg/L	<u>25 : 40</u> X 100 = <u> </u> %	<u>15 : 15</u> X 100 = <u>100</u> %

FETAX MALFORMATION DATA

Investigator	For	Test Material	2-AAF
Date	11/12/96	Test Number	1

MALFORMATION	CONCENTRATION															TOTAL
	F	F	6A	5A	5B	0.02	0.03	0.02	0.03	0.03	0.04	0.04	0.05	0.09	0.09	
Severe																
Stunted																
Gut																
Edema																
Multiple																
Cardiac																
Abdominal																
Facial																
Cephalic																
Optic																
Tail																
Notochord																
Fin																
Face																
Eye																
Brain																
Hemorrhage																
Cardiac																
Blisters																
Other-specify																
No. Malformed	0	0	18	15		5	10	19	26	38	40	40	40	39	36	32
Total No.	40	40	40	15		40	40	40	40	40	40	40	40	39	36	32

Comments:

Investigator	Ford	Test Material	2-AAF
Date	11/12/96	Test Number	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
FETAX							0 0	0	
FETAX							0 0	0	
5.5 b-AN							0 0	0	
2500 b-AN							15 10	25	
0.02							0 0	0	
0.025							0 0	0	
0.03							0 0	0	
0.035							0 0	0	
0.04							0 0	0	
0.045							0 0	0	
0.05							0 0	0	
0.09							0 1	1	
0.095							2 2	4	
0.1							5 3	8	
0.125							20 19	39	
0.15							20 20	40	

FETAX SUMMARY SHEET

Test Material <u>TCE</u>		Investigator <u>Fort</u>
Source <u>Alrich/Sigma</u>		Lab <u>SA</u>
CAS No.	Lot No.	Test Start Date
Composition/Purity		Test End Date
Solvent	Conc.	Test Units (i.e., mg/ml) <u>mg/mL</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u> </u> pH <u> </u>					
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %		
	<u>0</u> : <u>80</u> X 100 = <u>0</u> %	<u>0</u> : <u>80</u> X 100 = <u>0</u> %
Solvent Control	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀ <u>0.49</u>		EC ₅₀ <u>0.051</u>	
95% Confidence limits <u>0.48-0.50</u>		95% Confidence Limits <u>0.049-0.052</u>	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀)

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %
2500 mg/L	<u> </u> : <u> </u> X 100 = <u> </u> %	<u> </u> : <u> </u> X 100 = <u> </u> %

Investigator	Fort	Test Material	TCE
Date	11/12/96	Test Number	1

[illegible]

FETAX MALFORMATION DATA

Investigator	For	Test Material	TCB
Date	11/12/96	Test Number	1

MALFORMATION	CONCENTRATION																TOTAL	
	F	F	5.5	2500	0.035	0.01	0.045	0.05	0.055	0.06	0.075	0.13	0.35	0.4	0.46	0.5		0.55
Severe																		
Stunted																		
Gut																		
Edema																		
Multiple																		
Cardiac																		
Abdominal																		
Facial																		
Cephalic																		
Optic																		
Tail																		
Notochord																		
Fin																		
Face																		
Eye																		
Brain																		
Hemorrhage																		
Cardiac																		
Blisters																		
Other-specify																		
No. Malformed	0	0	16	14		0	2	10	18	21	38	40	40	31	33	27	—	—
Total No.	40	40	40	14		40	40	40	40	40	40	40	40	37	33	27	—	—
Comments:																		

Comments:

FETAX SUMMARY SHEET

Test Material <u>Conmarin</u>		Investigator <u>Fort</u>
Source <u>Sigma</u>	<u>SBR Army</u>	Lab <u>SA</u>
CAS No.	Lot No.	Test Start Date
Composition/Purity		Test End Date
Solvent	Conc.	Test Units (i.e., mg/ml) <u>mg/L</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>pH</u>					
Stock					
Control					
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number		
X 100 = %		
Solvent Control		
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	<u>168.74</u>	EC ₅₀	<u>59.79</u>
95% Confidence limits	<u>154.17 - 184.16</u>	95% Confidence Limits	<u>58.52 - 61.08</u>
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L		
2500 mg/L		

Investigator	Font	Test Material	Sumarin
Date	11/12/96	Test Number	1

Concentration	Stock (ml)	FETAX (ml)	24 hr	48 hr	72 hr	96 hr	Total	No	%
FETAX									
FETAX									
6-AN 5.5									
6-AN 2500									
0.045							↓ ↓ ↓ ↓ ↓	0	
0.05							↓ ↓ ↓ ↓ ↓	↓	
0.055							↓ ↓ ↓ ↓ ↓	↓	
0.06							↓ ↓ ↓ ↓ ↓	↓	
0.065							↓ ↓ ↓ ↓ ↓	↓	
0.07							↓ ↓ ↓ ↓ ↓	↓	
0.075							↓ ↓ ↓ ↓ ↓	↓	
0.095							↓ ↓ ↓ ↓ ↓	↓	
0.1							↓ ↓ ↓ ↓ ↓	↓	
0.15							↓ ↓ ↓ ↓ ↓	↓	
0.12							↓ ↓ ↓ ↓ ↓	↓	
0.25							↓ ↓ ↓ ↓ ↓	↓	

FETAX MALFORMATION DATA

Investigator	Fort	Test Material	Commanin
Date	11/12/96	Test Number	1

MALFORMATION	CONCENTRATION												TOTAL
	0	0.005	0.01	0.02	0.05	0.1	0.2	0.5	1	2	5	10	
Severe	0	0	0	0	0	0	0	0	0	0	0	0	
Stunted	0	0	0	0	0	0	0	0	0	0	0	0	
Gut	0	0	0	0	0	0	0	0	0	0	0	0	
Edema	0	0	0	0	0	0	0	0	0	0	0	0	
Multiple	0	0	0	0	0	0	0	0	0	0	0	0	
Cardiac	0	0	0	0	0	0	0	0	0	0	0	0	
Abdominal	0	0	0	0	0	0	0	0	0	0	0	0	
Facial	0	0	0	0	0	0	0	0	0	0	0	0	
Cephalic	0	0	0	0	0	0	0	0	0	0	0	0	
Optic	0	0	0	0	0	0	0	0	0	0	0	0	
Tail	0	0	0	0	0	0	0	0	0	0	0	0	
Notochord	0	0	0	0	0	0	0	0	0	0	0	0	
Fin	0	0	0	0	0	0	0	0	0	0	0	0	
Face	0	0	0	0	0	0	0	0	0	0	0	0	
Eye	0	0	0	0	0	0	0	0	0	0	0	0	
Brain	0	0	0	0	0	0	0	0	0	0	0	0	
Hemorrhage	0	0	0	0	0	0	0	0	0	0	0	0	
Cardiac	0	0	0	0	0	0	0	0	0	0	0	0	
Blisters	0	0	0	0	0	0	0	0	0	0	0	0	
Other-specify	0	0	0	0	0	0	0	0	0	0	0	0	
No. Malformed	0	0	0	0	0	0	0	0	0	0	0	0	
Total No.	40	40	40	40	40	40	40	40	40	40	40	40	

Comments:

Appendix B

Metabolic Activation System Test Kit Design

Version 4

FETAX Microsome Kit

Contents:

- ☐ Aroclor 1254-induced ☐ Isoniazid-induced ☐ Phenobarbital-induced
- ☐ β - Naphthoflavone-induced ☐ Phenobarbital- β - Naphthoflavone-induced 1:1 mix
- ☐ Aroclor 1254-induced-Isoniazid-induced 1:1 mix
- ☐ Phenobarbital, β - Naphthoflavone-induced- Isoniazid-induced 1:1 mix

Lot # _____ Preparation date _____

When a mix is checked it means that a lot of microsomes from a rat induced with one substance is mixed with another lot of microsomes from a rat induced with another substance. The 1:1 means that the activities have been adjusted to the proper number of units of activity according to the Nash assay. Mixed microsomes offer the ability to cover a wider range of metabolic activity than a single broad spectrum inducer.

Microsome Specific Activity _____ Units/mg protein

Protein Concentration of Microsomes in Kit _____ μ g/ml

BioRad Protein Assay run with IgG standard

performed by _____ date _____

Nash Units of microsomes in Kit _____ U/ml

performed by _____ date _____

FETAX

Positive Controls Date Performed _____ Technician _____

Microsome Quality Control:

FETAX positive control for Aroclor-induced microsomes.

Experiment	% Mortality	% Malformation
FETAX Controls		
4 mg/ml Cyclophosphamide		
4 mg/ml Cyclophosphamide + Microsomes		

Cyclophosphamide with Aroclor 1254-induced-microsomes should result in 50% mortality at the end of the 96 hrs.

Technician performing Assay _____ Date: _____

FETAX positive control for Isoniazid-induced microsomes

Experiment	% Mortality	% Malformation
FETAX Controls		
4 mg/ml Acetyl hydrazide		
4 mg/ml Acetyl hydrazide + Microsomes		

Acetyl hydrazide with isoniazid-microsomes should result in 50% mortality at the end of the 96 hrs.

Technician performing Assay _____ Date: _____

FETAX positive control for Aroclor-1254 induced and Isoniazid-induced (mixed) microsomes or Phenobarbital, β - Naphthoflavone-induced- Isoniazid-induced 1:1 mix

Experiment	% Mortality	% Malformation
FETAX Controls		
4 mg/ml Cyclophosphamide		
4 mg/ml Cyclophosphamide + Microsomes		

Experiment	% Mortality	% Malformation
FETAX Controls		
4 mg/ml Acetyl hydrazide		
4 mg/ml Acetyl hydrazide + Microsomes		

Cyclophosphamide with Aroclor 1254-induced-microsomes should result in 50% mortality at the end of the 96 hrs.

Acetyl hydrazide with isoniazid-microsomes should result in 50% mortality at the end of the 96 hrs.

Technician performing Assay _____ Date: _____

Storage:

Box 1-Microsomes: These must be stored at -80°C . If such a freezer is not available remove the tubes from the box and freeze under liquid nitrogen. There is one tube for each day of the experiment. Discard any left over microsomes and do not refreeze.

Box 2-Generator, glucose 6-phosphate dehydrogenase, cyclophosphamide (Aroclor 1254 positive control) and acetyl hydrazide (isoniazid positive control). Store frozen at -20°C .

Kit Design:

Each kit is designed to allow the contents to be directly pipeted into a 50 ml Erlenmeyer flask without further calculation. The total volume in the flask is 20 ml regardless whether glass or plastic dishes are being used. Generally, plastic dishes are used to help maintain sterility. However, autoclaved glass dishes may be used when the test material binds to plastic. Simply discard the remainder and thaw the contents of new tubes on each successive day of the test.

Thawing instructions:

Bring tubes to room temperature and with gentle rocking motion of the wrist mix the contents of the tube. Freezing often causes a salting out effect thereby requiring that the tube contents be resuspended in solution.. As soon as the last ice melts, place the tube in an ice bucket. Never shake or vortex a tube to mix contents, always stir or gently rock into solution. This is especially true for the microsomes. Frothing indicates denaturation of protein which means enzymatic function is lost.

Adding contents to the 50 ml Flask: Use an appropriate Pipetman for small quantities and use a disposable plastic Pipet for larger volumes.

1. If appropriate, add correct amount of cyclophosphamide to flask or acetyl hydrazide.
2. If appropriate, add test compound.
3. Calculate the amount of additional FETAX-AB needed to reach 20 ml. Add this amount and gently swirl to mix.
4. Add correct amount of Generator to flask.
5. Add correct amount of microsomal protein to flask.
6. Add correct amount of Glucose-6-Phosphate Dehydrogenase to flask.
7. The add 10 ml to each glass dish or 8 ml to each plastic dish as needed. Discard any remaining solution or use it for chemical analysis.

Kit Contents:

Component	Amount to Add/day	day 0	day 1	day 2	day 3	Kit
	(μ l)	Total Quantity (ml)	Total Quantity (ml)	Total Quantity (ml)	Total Quantity (ml)	Grand Total (ml)
Generator	192.5	6.3	6.3	6.3	6.3	25.2
Glucose-6-Phosphate dehydrogenase	62	2	2	2	2	8
Microsomal Protein	191	6.3	6.3	6.3	6.3	25.2
Cyclophosphamide	10,000	21	21	21	21	84
Acetyl hydrazide						

* Each kit holds enough material for 32 Petri dishes that contain MAS. There will always be some material left over.

Kit Supplies Materials to Prepare the Following Dishes:Dishes in an experiment that do not contain MAS.

FETAX solution only - 4 dishes

FETAX-AB solution - 2 dishes

FETAX solution plus 4 mg/ml cyclophosphamide (part of positive control)-2 dishes.

Dishes in an experiment that contains MAS.

FETAX- generator- microsomal protein (negative control)- 2 dishes.

FETAX- generator- microsomal protein treated with carbon monoxide-dithionite- 4 mg/ml cyclophosphamide (negative control)- 2 dishes.

FETAX- generator- microsomal protein + LC50 of test compound (control for interaction between MAS and test compound)- 2 dishes.

FETAX- generator- microsomal protein + EC50 of test compound (control for interaction between MAS and test compound)- 2 dishes.

FETAX- 4 mg/ml cyclophosphamide- generator- microsomal protein (part of positive control for Aroclor-induced microsomes)- 2 dishes.

FETAX- generator- (negative control for generator- no microsomal)- 2 dishes.

For different concentrations of the test material:

FETAX-generator-microsomal protein-test compound-2 dishes per test concentration (20 total).

Total dishes requiring MAS-32.



DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
FORT DETRICK, MARYLAND 21702-5012

REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y)

4 Dec 02

MEMORANDUM FOR Administrator, Defense Technical Information
Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir,
VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl


PHYLLIS M. RINEHART
Deputy Chief of Staff for
Information Management

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