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13. ABSTRACT (Maximum 200) This interlaboratory study of the Frog Embryo Teratogenesis Assay (FETAX) was undertaken in order to assess the repeatability and reliability of data collected under the guide published by the American Society for Testing and Materials. Another goal was to test 13 chemicals specified by the National Toxicology Program (NTP) of the National Institute of Environmental Health Sciences. FETAX is a developmental toxicity screening test useful for detecting environmental toxicants. The entire study was divided into 3 phases and the third phase was divided into 3 parts. Phases I, II and Phase III, part 1 were covered previously. The scope of this report is Phase 3-Part 2 covering the repeatability and reliability of the Aroclor 1254-induced metabolic activation system (MAS) with FETAX and Phase III-part 3 which concerns the testing of 13 chemicals for NTP. One chemical, 1,2 dichloroethane, was extremely volatile. Results for this chemical were deleted from this report since the report is in journal format. Phase III, part 2 showed that FETAX could yield repeatable results for one proteratogen and one chemical that was deactivated by MAS. Phase III, part 3 provided developmental toxicity data on twelve compounds that had previously been tested in mammals. Results indicated that FETAX could be used in testing for developmental toxicants.				
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John A. Bantle 6/10/97
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Table of Contents

Front Cover	1
SF 298, Report Documentation Page	2
Foreword	3
Table of Contents	4
Executive Summary	8
 Chapter I - Phase III, Part II	
Title Page; List of Participants	11
Abstract	11
Introduction	12
Methods and Materials	13
Interlaboratory Study Design & Protocol	13
Rat Liver Microsome Preparation	13
Animal Care & Breeding	14
Test Compounds & Assay Protocol	14
Data Analysis	16
Results	17
Control Results	17
Cyclophosphamide	17
Caffeine	18
Discussion	19
Acknowledgements	22
References	22
Tables	
Table 1 Interlaboratory Study Results for Cyclophosphamide	26
Table 2 Interlaboratory Study Results for Caffeine	27

Appendix

Interlaboratory Study of FETAX Phase III-Part 2	28
Cyclophosphamide - data summary sheets	29
Caffeine - data summary sheets	58

Chapter II - Phase III, Part III

Title Page; List of Participants	84
Abstract	85
Introduction	86
Methods and Materials	89
Interlaboratory Study Design & Protocol	89
Rat Liver Microsomes	89
Animal Care and Breeding	90
Test Compounds and Assay Protocol	90
Criteria for Judging Developmental Hazard	92
Data Analysis	94
Results	95
Variability and Teratogenic Hazard Data	95
Sodium Arsenite	95
Variability	95
Teratogenic Hazard	96
Boric Acid	96
Variability	96
Teratogenic Hazard	97
Ethylene Glycol	98
Variability	98
Teratogenic Hazard	99
Glycerol	100
Variability	100
Teratogenic Hazard	100

DTIC QUALITY INSPECTED 3

Sodium Iodacetate	101
Variability	101
Teratogenic Hazard	102
Acrylamide	103
Variability	103
Teratogenic Hazard	103
Triethylene Glycol Dimethylether	104
Variability	104
Teratogenic Hazard	105
Diethyleneglycol	106
Variability	106
Teratogenic Hazard	106
Phthalic Acid	107
Variability	107
Teratogenic Hazard	108
Dichloroacetate	109
Variability	109
Teratogenic Hazard	109
Sodium Bromate	110
Variability	110
Teratogenic Hazard	111
Tribromoacetic Acid	112
Variability	112
Teratogenic Hazard	112
Discussion	114
Variability	114
Intralaboratory	114
Interlaboratory	115
Predictive Ability	116
Effect of the Metabolic Activation System	117
Decision Criteria	117
Comparison to Mammalian Data	120
Relationship of Phase III-Part 3 Compounds	120
FETAX	120
FETAX Prediction	120

References	124
Tables	131
List of Chemicals	132
Table 1. Intralaboratory Data for Sodium Arsenite	133
Table 2. Intralaboratory Data for Boric Acid	134
Table 3. Intralaboratory Data for Ethylene Glycol	135
Table 4. Intralaboratory Data for Glycerol	136
Table 5. Intralaboratory Data for Sodium Iodoacetate	137
Table 6. Intralaboratory Data for Acrylamide	138
Table 7. Intralaboratory Data for Triethylene Glycol Dimethylether	139
Table 8. Intralaboratory Data for Diethylene Glycol	140
Table 9. Intralaboratory Data for Phthalic Acid	141
Table 10. Intralaboratory Data for Dichloroacetate	141.1
Table 11. Intralaboratory Data for Sodium Bromate	142
Table 12. Intralaboratory Data for Tribromoacetic Acid	143
Table 13. Results of the Phase III-Part3 Interlaboratory Study	144
Table 14. Estimation of Teratogenic Hazard for Each Chemical	145
Table 15. Interlaboratory Endpoint Variability based on C.V. Values	146
Table 16. FETAX Teratogenic Values Recorded During Validation.....	147
Data Summary Tables	148
Sodium Arsenite	149
Boric Acid	164
Ethylene Glycol	179
Glycerol	194
Sodium Iodoacetate	210
Acrylamide	225
Triethylene Glycol Dimethylether	240
Diethylene Glycol	255
Phthalic Acid	270
Dichloroacetate	285
Sodium Bromate	300
Tribromoacetic Acid	315
Bibliography and Personnel List	329

EXECUTIVE SUMMARY

FETAX (Frog Embryo Teratogenesis Assay- *Xenopus*) is a 96-h whole embryo developmental toxicity test that utilizes the embryos of the South African clawed frog, *Xenopus laevis*. A chemical is developmentally toxic when it causes harm to the embryo at concentrations too low to cause maternal toxicity. Basic FETAX endpoints are mortality (96-h LC50), 96-h EC50 (malformation)) and growth (Minimum Concentration that Inhibits Growth-MCIG) at 96 h of development. The types and severity of malformations caused are recorded because they often mimic results in mammals. FETAX was initially designed as an indicator of potential human developmental health hazards and this use has been enhanced by the development of an *in vitro* metabolic activation system (MAS) using Aroclor 1254- and/or isoniazid-induced rat liver microsomes. MAS is a substitute for the human liver which deactivates many toxicants and activates others before they are transported to the fetus. The mammalian fetus does not have these enzymes functioning and relies on maternal systems.

FETAX is designed to be part of an integrated bioassessment battery of subchronic tests that employ alternatives to mammalian systems to test for environmental toxicants harmful to humans. This battery covers weak or highly specialized links in the life cycle such as reproduction, development, neurotoxicity, immunotoxicity, acute toxicity, cancer, etc. It makes extensive use of higher vertebrates such as fish or frogs which are easily cultured, cheap and available in sufficient numbers to make statistical analysis easy. Using higher vertebrates such as frogs and fish ensures that basic developmental processes, structure and biochemistry are at least close to mammals. The short time period for the assays is a real advantage in defraying cost and helping to speed testing compared to mammalian assays. From time, cost, animal rights and statistical considerations, the assays provide an excellent alternative to mammals.

FETAX is also applicable to aquatic toxicity assessments. It is well suited for structure-activity relationship analysis, and useful in the testing of complex mixtures such as industrial effluents or mixtures found at hazardous waste sites. Recent modifications have been made to FETAX to allow routine testing of volatile organics, soils and sediments. FETAX has also been used to evaluate the success of remediation efforts.

FETAX may help in studies designed to discover the reasons for the reported world-wide disappearance of amphibians even in pristine locations. Although the problem is complex, the role of FETAX in this area is to investigate the extent and causes of the decline when attributable to environmental degradation.

An American Society for Testing and Materials *New Standard Guide for the Conduct of FETAX* was published along with a companion *Atlas of Abnormalities* that aids in embryo staging and identifying malformations. A book chapter in *Fundamentals of Aquatic Toxicology* (2nd ed.) has been published that provides additional

experimental protocols and rationale for the assay. The publication of these protocols and guides necessitated the present study.

As part of the ASTM process, an interlaboratory validation study (ILS) was undertaken to determine the repeatability and reliability of FETAX. Repeatability is the variation encountered when the same laboratory repeats the test using the same protocol. Reliability is the measure used when comparing results between laboratories. To be useful, an assay must be able to perform in many different laboratories always yielding the same results. Secondary goals were the improvement of the FETAX protocol and the testing of additional compounds whose mammalian developmental toxicity was known. In accordance with the ASTM recommendations for ILS studies, a three phase experimental plan with seven participants was designed. The phases allow for training and for protocol changes to be worked into the study. This keeps the investigator from performing many expensive tests that ultimately prove to be too variable to be useful. It also shows the sources of variability so protocol changes can be made. Phase I was a training and protocol evaluation phase in which the identity of the three test materials was known. Because they had previously been tested in FETAX, the same concentrations needed to establish the 96-h LC50 and EC50 (malformation) were used by all laboratories. Phase I showed that proper technician training was important in obtaining repeatable and reliable data. Several protocol changes were necessitated because of Phase I. For example the 6-aminonicotinamide positive control was designed to show that a laboratory was performing the assay correctly. This portion of the protocol was heavily modified. Additionally, a new way of more accurately mixing the test chemicals with the buffer solution was devised. Phase I results in terms of variability and correspondence to historical data were very good with only occasional high variation observed in some laboratories. Phase II was designed to be similar to Phase I except that the identity of the test materials was not known. All technicians had greater experience in Phase II than in Phase I and the nature of the test compounds may have played a role in the excellent results obtained in Phase II. Phase II showed far less intra- and interlaboratory variability than Phase I. Nonteratogens showed the most consistent results while more variability was observed for the two teratogens tested. Interlaboratory coefficient of variation values for all FETAX endpoints ranged from 7.3 to 54.7%. Ordinarily values approaching 75% are considered very good and as high as 150% acceptable. The most variable endpoint was the MCIG and the least variable was the LC50. Phase III was supported by this contract. Phase III-Part 1 was reported as part of the mid-term report while this final report covers, Phase III- Parts 2 and 3.

Phase III-Part 1 was designed to test FETAX using six test compounds in a blind testing format. Each laboratory determined the concentrations to be tested but did not know the identity of the compound. Results indicated that although generally acceptable data were obtained, a new protocol for range finding was needed so that repeatable and reliable results could be obtained. This was the first time test concentrations had not been given to participants and some chose wide concentration ranges while others chose very narrow ranges. This contributed to excessive variability.

It was also observed that some technicians graded malformations more severely than others. The *in vitro* metabolic activation system consisting of Aroclor or isoniazid-induced rat liver microsomes for FETAX was not employed in either Phases I, II or III-Part 1. In Phase III-Part 2, two compounds were tested using a metabolic activation system employing Aroclor 1254-induced rat liver microsomes. The experimental design approximated Phase II when the compounds had previously been tested in FETAX and the test concentrations provided to each laboratory. The samples were coded and contained one compound activated by the microsomes and one that was deactivated. Results indicated that excellent, although slightly more variable results, could be obtained when the embryos were co-cultured with the MAS.

In the Phase III-part 3 study, three laboratories, all very experienced in performing FETAX, tested 12 coded chemicals with and without Aroclor 1254-induced microsomes and each laboratory was responsible for determining tests concentrations. This last phase closely approximated real world testing in that a laboratory would receive an unknown test sample and have to devise a research plan complete with a selection of concentrations to be tested complete with MAS addition. A stringent decision table was then constructed based on the results of Phase III-Part 3. A decision table allows a prediction to be made when the data indicates certain criteria have been met. Because the compounds selected for Phase III - Part 3 were either non-teratogens or weak teratogens, they were helpful in establishing decision table criteria. Four of the test compounds were non-teratogens and two of the chemicals were clearly teratogenic in nature while the remaining six were in between. Reference to the types and severity of malformations caused aided in classification. The results were discussed in light of the difficulty of producing an adequate decision table. FETAX proved to yield repeatable and reliable data as long as care was taken during range finding and the technicians were adequately trained. The metabolic activation system was essential in using FETAX to predict developmental hazard in mammals although it still requires further development and standardization.

**Phase III INTERLABORATORY STUDY OF FETAX, PART 2:
INTERLABORATORY VALIDATION OF AN EXOGENOUS
METABOLIC ACTIVATION SYSTEM FOR
FROG EMBRYO TERATOGENESIS ASSAY - *XENOPUS* (FETAX)**

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ABSTRACT

Interlaboratory validation of an exogenous metabolic activation system (MAS) developed for the alternative, short-term developmental toxicity bioassay, Frog Embryo Teratogenesis Assay - *Xenopus* (FETAX) was performed with cyclophosphamide and caffeine. Seven study groups within six separate laboratories participated in the study in which three definitive concentration-response experiments were performed with and without the MAS in a side-by-side format for each chemical. Since both chemicals had been previously tested in FETAX, the test concentrations were provided to each laboratory prior to testing. Interlaboratory coefficient of variation (CV) values for unactivated cyclophosphamide (no MAS) were 15%, 15%, 29%, and 25% for the 96-h LC50, 96-h EC50 (malformation), Minimum Concentration to Inhibit Growth (MCIG), and Teratogenic Index (TI) values, respectively. Addition of the MAS increased the CV values of each endpoint at least 3.9-fold. Interlaboratory CV values for unactivated caffeine were 31%, 18%, 31%, and 46% for the 96-h LC50, 96-h EC50 (malformation), MCIG, and TI values, respectively. Addition of the MAS decreased the CV values of each respective endpoint by at least 1.6-fold. Results

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indicated that bioactivated toxicants may be prone to greater variability in response amongst laboratories than compounds which are detoxified. Even though more variability was noted with activated cyclophosphamide, results were within interlaboratory variation expected for other aquatic-based bioassays. Thus, results from these studies warrant the continued use and further refinement of FETAX for alternative developmental toxicity assessment.

INTRODUCTION

Alternative bioassays, such as the Frog Embryo Teratogenesis Assay - *Xenopus* (FETAX), provide a rapid, simple, and cost-effective means of evaluating development toxicants. FETAX is a 4-d, whole-embryo, assay utilizing embryos of the South African clawed frog, *Xenopus laevis*.¹ Development², validation³, and optimization⁴ of a complimentary exogenous metabolic activation system (MAS) have increased the utility of FETAX since early *Xenopus* embryos lack many major detoxification pathways. In addition, the exogenous MAS co-cultured with FETAX has been successfully used to elucidate the toxicological mechanisms of nicotine⁵, diphenylhydantoin⁶, isoniazid⁷, acetaminophen⁸, and trichloroethylene⁹ *in vitro*.

An American Society of Testing and Materials (ASTM) Standard Guide for the conduct of FETAX¹⁰ and a companion user's document, Atlas of Abnormalities¹¹, have been prepared. As a formal component of the ASTM process, a three-phase interlaboratory validation study was initiated to determine the repeatability and reliability of FETAX. During the first phase, each participating laboratory was trained to perform the FETAX assay so that a pilot study could be performed. Results from Phase I suggested that FETAX was more repeatable and reliable than several other bioassays.¹² Reduced variability in results obtained during blind Phase II studies in which the test concentrations were provided to the laboratories suggested that an initial lack of experience may

have been the cause of the increased variability noted in the initial phase.¹³ Phase III studies¹⁴ which included a completely blind format with no test concentrations provided, as well as an assessment of the exogenous MAS, showed similar but slightly higher levels of variability noted in the previous phases. Results of initial interlaboratory studies with the exogenous MAS are described in this report.

MATERIALS AND METHODS

Interlaboratory Study Design and Protocol

Each laboratory participating in this interlaboratory study included a principal investigator and, with a single exception, one or two primary technicians. Six different laboratories participated in the study of cyclophosphamide and five for experiments with caffeine. Each laboratory utilized one technician with the exception of one laboratory, which utilized two independent technicians. For ease of discussion, we will refer to seven and six laboratories being used, respectively. The technicians performed FETAX while the principal investigators compiled and reported the data to a central coordinator. The experimental results were screened by the coordinator to see that they complied with the standard protocol established in the ASTM Standard Guide.¹⁰

Rat Liver Microsome Preparation

Aroclor 1254-induced rat liver microsomes were prepared as described previously.² Five days prior to microsome preparation, male Sprague-Dawley rats were injected with 500 mg/Kg Aroclor 1254 i.p. in corn oil.¹⁵ Microsomal P-450 activity was estimated by measuring the N-demethylation of aminopyrine to formaldehyde using the methods of Lucier et al.¹⁶ and Nash¹⁷ as described previously.² Protein was determined by the method of Bradford¹⁸ (BioRad®, Richmond, CA). All rat liver microsomes were prepared by Dr. Bantle's

laboratory and shipped frozen on dry ice to each of the participating laboratories. Prior to shipment, each lot of microsomes was diluted with Tris-HCl buffer (pH 7.5) to an appropriate N-demethylase activity so that each laboratory would effectively use the same activity throughout the study. To verify further the activity, each lot of microsomes was tested by co-culturing with embryos and 4.0 mg/mL cyclophosphamide which should induce 100% embryo lethality. All laboratories used the same lots of microsomes for each test compound to minimize interlaboratory variability. Following arrival, each laboratory stored the microsomes in liquid nitrogen until needed.² Specific aliquots of the Aroclor 1254-induced microsomes were pretreated with carbon monoxide (CO) to selectively inhibit enzyme activity. Each laboratory was responsible for preparing their own CO-inactivated microsomes. Inhibition with CO gas was achieved by chemically reducing designated aliquots with dithionite and subsequent bubbling of CO through the reconstituted microsomes for 5 minutes.²

Animal Care and Breeding

Xenopus culture, breeding procedures and egg sorting were described previously in the ASTM Standard Guide¹⁰ and the Atlas of Abnormalities.¹¹ Adult *Xenopus laevis* frogs were obtained from Xenopus I (Ann Arbor, MI) or Xenopus Express (Beverly Hills, FL).

Test Compounds and Assay Protocol

Chemicals tested in this portion of the Phase III interlaboratory validation study were cyclophosphamide and caffeine. The chemicals were purchased from Sigma Chemical Company (St. Louis, MO) in bulk quantities from the same lot. One technician individually weighed the compounds using a calibrated analytical balance. The amount was then placed in 100-mL serum vials (Fisher Scientific,

Houston, TX), capped with rubber septa, and sealed with aluminum lids. Each vial contained enough material to prepare test solutions for each 24-h interval of the experiment. Four vials were needed for each test. The chemicals were sent to a coordinator who coded the samples and then shipped enough chemical to each laboratory for three definitive concentration-response experiments. Instructions shipped with each chemical included: (a) the stock concentration, (b) test concentrations, (c) concentrations from which to take samples for chemical analysis, and (d) a Material Safety Data Sheet, sealed in an envelope which was available for use in an emergency.

Tests were performed as specified in the ASTM Standard Guide.¹⁰ All technicians used the same concentration ranges but did not know which chemical they were testing as the samples were coded.

Samples of test solutions were retained from selected concentrations and were frozen in specially supplied borosilicate glass tubes with Teflon-lined lids (Fisher Scientific, Houston, TX). At the end of the experiment, samples from each laboratory were shipped frozen on dry ice to Dr. Bantle's laboratory for concentration analysis in the event that the cause of excess variability had to be identified. Embryos preserved in 3% v/v formalin were stored in glass scintillation vials (RPI, Elkhart, IL) and were available to be sent to Dr. Bantle's laboratory for verification of results when necessary.

For tests conducted without the MAS, groups of 20 embryos were placed in 60-mm covered, plastic Petri dishes (Fisher Scientific, Houston, TX) with varying constant concentrations of toxicant. Each toxicant vial received by the participating laboratories was reconstituted with FETAX Solution, a reconstituted water medium suitable for the culture of *Xenopus* embryos.¹⁹ Six to seven test

concentrations were tested in duplicate. Four separate dishes of 20 embryos were exposed to FETAX Solution alone and designated FETAX Solution (negative) controls. Each treatment was prepared in a 50 mL Erlenmeyer flask and each dish received a total volume of 8 mL of solution.

Tests performed with the MAS were also performed in duplicate with 20 embryos per replicate concentration. Each metabolically activated/inactivated treatment received 0.4 U/dish of aminopyrine N-demethylase activity, a NADPH generating system, and a 100 U/mL penicillin-streptomycin mixture mixed in the FETAX Solution to help control bacterial contamination. For each test, 9 to 11 concentrations were tested side-by-side with the unactivated (no MAS) tests. Controls including: FETAX Solution with antibiotics, MAS, activated cyclophosphamide (FETAX reference proteratogen), and cyclophosphamide co-incubated with CO-inactivated MAS were tested simultaneously with each experiment. All control treatments were prepared with FETAX Solution supplemented with antibiotics. Three separate concentration-response tests were performed by each laboratory with both test compounds.

Data Analysis

Probit analysis, using the method of Litchfield-Wilcoxon, was used to determine the 96-h LC50 (median lethal concentration), 96-h EC50 (concentration inducing malformations in 50% of the surviving embryos), and 95% confidence intervals. When the homogeneity test failed, either the trimmed Spearman-Kärber or EPA probit method was used instead of the Litchfield-Wilcoxon probit analysis. Teratogenic hazard was determined using a teratogenic index $[TI = LC50/EC50 \text{ (malformation)}]$. Head-tail length (growth) was measured using an IBM-compatible computer equipped with digitizing software (Jandel Scientific,

Corte Madera, CA). For each test, the minimum concentration to inhibit growth (MCIG) was calculated using the t-test for grouped observations ($P < 0.05$). The coefficient of variation (CV) values for the 96-h LC50, 96-h EC50, TI, and MCIG were calculated according to Steel and Torrie.²⁰

RESULTS

Control Results

Results for data reported from each laboratory met or exceeded the FETAX Solution quality control criteria specified in the ASTM Standard Guide (less than or equal to 10% mortality and malformation at 96h) for all tests except Laboratory 4 for cyclophosphamide, experiments 1 and 3. MAS, positive MAS, and negative MAS control results met or exceeded guidance limits set by previous studies²⁻⁴, <10% mortality and malformation, 100% mortality, and <50% mortality, respectively.

Cyclophosphamide

Results from the three concentration-response tests of cyclophosphamide by each of the seven laboratories are presented in Table 1. The 96-h LC50 values for unactivated cyclophosphamide ranged from 5.72 mg/mL to 9.74 mg/mL with interlaboratory mean and coefficient of variation values of 7.38 mg/mL and 15%, respectively. The 96-h EC50 (malformation) values for unactivated cyclophosphamide ranged from 3.73 mg/mL to 6.50 mg/mL with interlaboratory mean and coefficient of variation values of 4.92 mg/mL and 15%, respectively. MCIG values for unactivated cyclophosphamide ranged from 3.0 mg/mL to 6.0 mg/mL with interlaboratory mean and coefficient of variation values of 3.81 mg/mL and 29%, respectively. TI values ranged from 1.10 to 2.46 with

interlaboratory mean and coefficient of variation values of 1.54 and 25%, respectively.

For tests performed with the exogenous MAS, the mean 96-h LC50 value was 1.53 mg/mL ranging from 0.4 mg/mL to 4.15 mg/mL, with a coefficient of variation value of 53%. The mean 96-h EC50 (malformation), range, and coefficient of variation values were 0.61 mg/mL, 0.11 mg/mL to 1.51 mg/mL, and 64%, respectively. The mean, range, and coefficient of variation for the MCIG values were 0.38 mg/mL, 0.05 mg/mL to 2.0 mg/mL, and 131%, respectively. The mean TI value was 3.62 with an interlaboratory range and coefficient of variation value of 1.08 to 11.69, and 83%, respectively.

Caffeine

Results from the three separate concentration-response tests of caffeine by each of the six participating laboratories are presented in Table 2. The 96-h LC50 values of unactivated caffeine ranged from 0.23 mg/mL to 1.04 mg/mL with an interlaboratory mean value of 0.59 mg/mL and coefficient of variation value of 31%. The 96-h EC50 (malformation) values ranged from 0.09 mg/mL to 0.18 mg/mL with an interlaboratory mean and coefficient of variation value of 0.14 mg/mL and 18%. The MCIG mean, range, and coefficient of variation values for unactivated caffeine were 0.13 mg/mL, 0.10 mg/mL to 0.20 mg/mL, and 31%, respectively. TI mean, range, and coefficient of variation values for unactivated caffeine were 4.48, 1.77 to 9.44, and 47%, respectively.

The interlaboratory mean 96-h LC50 and associated range were 0.27 mg/mL, and 0.20 mg/mL to 0.37 mg/mL, respectively, for activated caffeine. The interlaboratory coefficient of variation for the 96-h LC50 was 18%. The 96-h EC50 (malformation) interlaboratory mean, range, and coefficient of variation

were 0.12 mg/mL, 0.09 mg/mL to 0.16 mg/mL, and 19%, respectively. The MCIG values ranged from 0.10 mg/mL to 0.18 mg/mL with an associated mean value of 0.11 mg/mL. The interlaboratory coefficient of variation for the MCIG values was 20%. The mean, range, and coefficient of variation values for the activated caffeine TI were 2.26, 1.38 to 2.90, and 21%, respectively.

DISCUSSION

The teratogenic potential of chemicals assayed with FETAX have been routinely assessed based on TI values, growth endpoints, and types and severity of induced terata.¹⁻¹⁴ In general, TI values <1.5 indicate low teratogenic potential, as little or no separation exists between the concentrations that induced malformation without embryo lethality and concentrations causing lethal effects. Thus, greater TI values represent greater separation between the malformation and mortality response curves and thus, a greater potential for embryos to be malformed, yet alive. However, other endpoints including, types/severity of abnormalities and growth inhibition, are also considered in the hazard evaluation.

Based on this form of teratogenic hazard evaluation, five of the seven laboratories consistently determined that the teratogenic potential of cyclophosphamide increased with the inclusion of the exogenous MAS. Based on the mean interlaboratory values, the study also suggested that bioactivation increased the teratogenic potential of cyclophosphamide in *Xenopus*. Inclusion of the MAS decreased the mean LC50, EC50, MCIG, and increased the TI values 4.8-fold, 8.1-fold, 10.0-fold, and 2.4-fold, respectively. The mean interlaboratory values were consistent with those values reported by Fort et al.²

Each of the six laboratories consistently determined the addition of the MAS decreased the potential teratogenic hazard of caffeine in *Xenopus*. Inclusion

of the MAS reduced the mean LC50 and TI values 2.2-fold and 2.0-fold, respectively, but altered the EC50 (malformation) and MCIG values insignificantly. Thus, the decreased teratogenic hazard caused by inclusion of the MAS appeared to be the result of increased embryo lethality. Results obtained in these studies with caffeine were comparable to those obtained by Dawson and Bantle.²¹ In general, the results obtained with both test compounds agreed with assessments performed with mammalian species.^{2, 21-35}

Metabolic activation of cyclophosphamide increased interlaboratory variability much more than with caffeine. Tests of caffeine with the exogenous MAS actually decreased interlaboratory variability. However, compared to Phase I and II studies, the variability was not excessive. Interlaboratory coefficients of variation for all endpoints ranged from 20.5% to 201.5% in Phase I¹³, to 8.7% to 44.8% in Phase II studies.¹⁴ Neither Phase I nor Phase II studies were performed with the exogenous MAS. Decreased variability observed in Phase II studies was attributed to greater technician experience. Only two of the participating laboratories had experience in using the exogenous MAS with FETAX prior to the present study. Furthermore, results from these present studies with the MAS were well within interlaboratory variability limits determined by Parkhurst et al.³⁶ for 48-h to 96-h aquatic toxicity tests with *D. magna*, *D. pulex*, *P. promelas*, *M. bahia*, and *C. variegatus*. The coefficients of variation ranged from 22% to 143% for these aquatic toxicity tests. Greater variability noted with the growth endpoint (MCIG) was not surprising since the concentrations selected for testing were based on both the lethal and malformation-inducing concentration curves, and not those concentrations inhibiting growth.

Other rationale to explain the greater interlaboratory variability observed with cyclophosphamide compared to caffeine include differences in metabolic complexities and toxicodynamics. Since FETAX can not simulate all of the toxicodynamic intricacies of *in vivo* mammalian test systems, greater variability associated with use of the MAS in FETAX would not necessarily be unexpected. The complexity of FETAX is increased by including the MAS. Cyclophosphamide is efficiently bioactivated by P-450 to 4-hydroxycyclophosphamide and eventually phosphoramidate mustard.³⁴ Differences in the amount of bioactivated toxicant produced in each experiment in each laboratory will cause substantial variability in the quantitative endpoints.² Caffeine, on the other hand, has a fairly complex metabolic pathway resulting in several metabolic by-products, each with comparable toxicities on an equimolar basis in *Xenopus*.²¹ Activated toxicant stabilities and the magnitude of change in the toxicities of cyclophosphamide and caffeine on an equimolar basis must also be considered. Cyclophosphamide activation by P-450 produces highly reactive metabolic products, whereas, P-450-mediated metabolism of caffeine produces fairly unreactive detoxification products, such as, 1,3 dimethyluric acid, 1-methylxanthine, and 3-methylxanthine.³⁷ Thus, since the toxicologically-active metabolic products of cyclophosphamide are more reactive, and thus short-lived, consistent embryo exposure is probably not occurring. In addition, the alteration in the developmental toxicity of activated cyclophosphamide (2.4 to 10.0-fold) compared to detoxified caffeine (ca. 2.0-fold) by the addition of the MAS may cause results with cyclophosphamide to be inherently more variable than with caffeine.

Overall, Phase III interlaboratory validation studies of FETAX and the exogenous MAS demonstrated the repeatability and reliability of the assay, especially after proper technician training and more experience with the system. Furthermore, these studies support the continued use of FETAX to identify developmental toxicants in the workplace and the environment on a routine basis.

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Table 1. Results of the Interlaboratory Study of Cyclophosphamide with and without Metabolic Activation System.

Laboratory	Replicate ¹	Without MAS					With MAS				
		LC50 ² (mg/mL)	EC50 ³ (mg/mL)	MCIG ⁴ (mg/mL)	TI ⁵	LC50 ² (mg/mL)	EC50 ³ (mg/mL)	MCIG ⁴ (mg/mL)	TI ⁵	LC50 ² (mg/mL)	EC50 ³ (mg/mL)
1	1	8.54	4.26	5.00	2.00	4.15	0.44	2.00	9.43	4.15	0.44
	2	8.20	6.50	6.00	1.26	1.86	----- ⁶	0.10	----- ⁶	1.86	----- ⁶
	3	8.02	6.16	6.00	1.30	2.12	0.29	0.25	7.31	2.12	0.29
2	1	8.73	3.73	3.00	2.34	1.52	0.13	0.10	11.69	1.52	0.13
	2	8.95	4.03	3.00	2.22	0.74	0.11	0.05	6.73	0.74	0.11
	3	9.74	3.96	3.00	2.46	1.01	0.17	0.05	5.94	1.01	0.17
3	1	6.53	4.41	3.00	1.48	1.12	1.04	0.10	1.08	1.12	1.04
	2	7.94	5.14	3.00	1.55	0.44	0.28	0.10	1.57	0.44	0.28
	3	7.71	5.45	3.00	1.42	0.40	0.31	0.05	1.29	0.40	0.31
4	1 ⁷	7.12	5.15	3.00	1.38	--- ⁶	--- ⁶	0.05	--- ⁶	--- ⁶	--- ⁶
	2	7.50	5.91	3.00	1.27	1.24	0.86	0.05	1.44	1.24	0.86
	3 ⁷	7.36	3.74	3.00	1.97	1.33	0.86	0.25	1.55	1.33	0.86
5	1	6.72	5.30	4.00	1.28	2.25	1.51	0.75	1.49	2.25	1.51
	2	6.32	4.85	4.00	1.30	2.51	1.22	1.00	2.06	2.51	1.22
	3	5.81	4.50	5.00	1.29	1.76	1.11	0.75	1.59	1.76	1.11
6	1	6.22	4.88	3.00	1.27	1.69	0.82	0.50	2.06	1.69	0.82
	2	6.12	4.86	3.00	1.26	1.23	0.53	0.50	2.32	1.23	0.53
	3	6.47	5.01	3.00	1.29	1.04	0.56	0.05	1.86	1.04	0.56
7	1	5.72	5.19	4.00	1.10	1.12	0.32	0.05	3.50	1.12	0.32
	2	7.35	5.34	4.00	1.38	1.62	0.53	1.25	3.06	1.62	0.53
	3	7.92	5.05	6.00	1.57	1.38	0.48	0.05	2.88	1.38	0.48
Mean (mg/mL)		7.38	4.92	3.81	1.54	1.53	0.61	0.38	3.62	1.53	0.61
Coefficient of Variation (%)		15	15	29	25	53	64	131	83	53	64

¹ Represents separate experiments performed by each laboratory.² 96-h median lethal effect concentration.³ 96-h median teratogenic concentration.⁴ Minimum concentration to inhibit growth, P<0.05.⁵ Teratogenic Index = 96-h LC50/96-h EC50 (malformation).⁶ Could not be calculated from the data.⁷ Control values exceeded those listed in ASTM Standard Guide for FETAX.

--Endpoint could not be calculated.

Table 2. Results of the Interlaboratory Study of Caffeine with and without Metabolic Activation System.

Laboratory	Replicate ¹	Without MAS					With MAS				
		LC50 ² (mg/mL)	EC50 ³ (mg/mL)	MCIG ⁴ (mg/mL)	TI ⁵	LC50 ² (mg/mL)	EC50 ³ (mg/mL)	MCIG ⁴ (mg/mL)	TI ⁵	LC50 ² (mg/mL)	TI ⁵
1	1	0.23	0.13	0.20	1.77	0.36	0.13	0.18	2.77		
	2	0.46	0.14	0.10	3.29	0.22	0.09	0.10	2.44		
	3	0.55	0.13	0.10	4.23	0.21	0.11	0.10	1.91		
2	1	0.85	0.09	0.10	9.44	0.28	0.12	0.10	2.33		
	2	0.85	0.10	0.10	8.50	0.28	0.10	0.10	2.80		
	3	1.04	0.13	0.15	8.00	0.32	0.12	0.10	2.67		
3	1	0.55	0.14	0.10	3.92	0.24	0.10	0.10	2.40		
	2	0.57	0.13	0.10	4.38	0.23	0.10	0.10	2.30		
	3	0.71	0.11	0.10	6.45	0.29	0.10	0.10	2.90		
4 ⁶	1	---	---	---	---	---	---	---	---		
	2	---	---	---	---	---	---	---	---		
	3	---	---	---	---	---	---	---	---		
5	1	0.53	0.16	0.20	3.31	0.31	0.16	0.15	1.94		
	2	0.53	0.17	0.20	3.12	0.25	0.15	0.15	1.67		
	3	0.49	0.13	0.15	3.77	0.20	0.12	0.12	1.67		
6	1	0.40	0.14	0.10	2.86	0.26	0.14	0.12	1.86		
	2	0.45	0.16	0.10	2.81	0.24	0.14	0.12	1.71		
	3	0.47	0.16	0.10	2.94	0.22	0.16	0.10	1.38		
7	1	0.57	0.18	0.10	3.17	0.29	0.10	0.10	2.90		
	2	0.67	0.13	0.10	5.15	0.26	0.10	0.10	2.60		
	3	0.63	0.18	0.10	3.50	0.37	0.15	0.10	2.47		
Mean (mg/mL)		0.59	0.14	0.13	4.48	0.27	0.12	0.11	2.26		
Coefficient of Variation (%)		31	18	31	47	18	19	20	21		

¹ Represents separate experiments performed by each laboratory.² 96-h median lethal effect concentration.³ 96-h median teratogenic concentration.⁴ Minimum concentration to inhibit growth, $P < 0.05$.⁵ Teratogenic Index = 96-h LC50/96-h EC50 (malformation).⁶ Did not participate in this particular study, but participated in earlier studies.^{12,13}

--Endpoint could not be calculated.

INTERLABORATORY STUDY OF FETAX

Phase III-Part 2

CYCLOPHOSPHAMIDE
DATA SUMMARY SHEETS
PHASE III-PART 2

Cyfluthrin
#1

FETAX Summary Sheet

Test No. 1 PART A

Test Material	P3M1	Investigator	JAMES RAYBURN
Source	SIGMA	Laboratory	BANTLE
CAS No.	Lot No.	Test Start Date:	AUG 2 1993
Composition/Purity		Test End Date	AUG 6 1993
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.4	7.4	7.4	7.3	
Control		8.4	8	7.4	7
Highest Concentration		7.2	6.6	6.6	6.5

No. Dead or Malformed		
----- X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	5 : 80 X 100 = 6%	7 : 75 X 100 = 9.3%
MAS CONTROL	3 : 40 X 100 = 7.5%	4 : 37 X 100 = 10.8%
Control Length	MAS Control Length	
Minimum Concentration to Inhibit Growth (MCIG)	5.0	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	5	N.A.	T-test
LOEL	6	3	T-test
LC50	8.541	EC50	4.256
95% CL	6.812 -- 10.709	95% Confidence limits	3.800 ---- 4.766
Test Teratogenic Index (TI = LC50/EC50):		2.01	
95% Confidence limits		1.56 -- 2.58	

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	: X 100 =	: X 100 =
2500 mg/L	: X 100 =	: X 100 =

CL = Confidence limits

Cyfluthrin 0.1613 = 1

FETAX Summary Sheet

Test No. 1 PART B

Test Material	P3M1	Investigator	JAMES RAYBURN
Source	SIGMA	Laboratory	BANTLE
CAS No.	Lot No. MIX3	Test Start Date:	AUG 2 1993
Composition/Purity		Test End Date	AUG 6 1993
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7.4	7.4	7.4	7.3	
Control		8.4	8	7.4	7
Highest Concentration		7.2	6.6	6.6	6.5

No. Dead or Malformed		
----- X 100 = %		
Total Number		
	Mortality Record	Malformation Record
FETAX Control	5 : 80 X 100 = 6%	7 : 75 X 100 = 9.3%
MAS CONTROL	3 : 40 X 100 = 7.5%	4 : 37 X 100 = 10.8%
Control Length	MAS Control Length	
Minimum Concentration to Inhibit Growth (MCIG)	2.0	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.05	0.05	T-test
LOEL	2	0.1	T-test
LC50	4.15	EC50	0.442
95% CL	2.33 -- 7.39	95% Confidence limits	0.245 ---- 0.797
Test Teratogenic Index (TI = LC50/EC50):		9.40	
95% Confidence limits		4.12 -- 21.45	

POSTIVE CONTROL: 6-AMINONICOTINAMIDE (6-AN) RESULTS

Concentration	Mortality	Malformation
5.5 mg/L	: X 100 =	: X 100 =
2500 mg/L	: X 100 =	: X 100 =

CL = Confidence limits

Cyclophosphamide

James Rayburn

Cyclophosphamide

James Rayburn

Data Sheets Follow

Optiphenolide w/ PKAFS #3

FETAX Summary Sheet

Test No. 3

Test Material	P3M1 unactivated	Investigator	James Rayburn
Source		Laboratory	Bantle
CAS No.	Lot No.	Test Start Date:	July 13, 1994
Composition/Purity		Test End Date	July 17, 1994
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	6.8	7	6.8	6.8	
Control		7.7	7.6	7.5	7.2
Highest Concentration		6	6.3	6.4	6.4

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length (mm) 9.4

Mortality Record

Malformation Record

5 : 80 X 100 = 6% 7 : 75 X 100 = 9.3%

: X 100 = : X 100 =

Solvent Control Length (mm)

Minimum Concentration to Inhibit Growth (MCIG) 6 mg/ml

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	6	N.A.	T-test
LOEL	9	6	T-test
LC50	8.020	EC50	6.155
95% CL	7.610 -- 8.453	95% Confidence limits	5.825 ---- 6.503

Test Teratogenic Index (TI = LC50/EC50): 1.30

95% Confidence limits 1.21 -- 1.41

Percent effect	LC	EC
5	6.4722512	5.658
16	7.0449019	5.849
50	8.0199981	6.155
84	9.1300591	6.476
95	9.9378668	6.695

Exp 1 - 1st - 2nd w/ 10.12 #3

FETAX Summary Sheet

Test No. 3

Test Material	P3M1 activated	Investigator	James Rayburn
Source		Laboratory	Bantle
CAS No.	Lot No.	Test Start Date:	July 13, 1994
Composition/Purity		Test End Date	July 13, 1994
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	6.8	7	6.8	6.8	
Control		7.7	7.6	7.5	7.2
Highest Concentration		6	6.3	6.4	6.4

No. Dead or Malformed	Mortality Record		Malformation Record	
X 100 = % FETAX Con	5 : 80	X 100 = 6%	7 : 75	X 100 = 9.3%
Total Number MAS control	3 : 40	X 100 = 7.5%	3 : 37	X 100 = 8.1%
CP 4 mg/l Postive CP	40 : 40	X100 = 100.0%	:	X 100 =
CP 4 mg/l Negative CP	3 : 40	X 100 = 7.5%	5 : 37	X 100 = 13.5%
Control Length (mm)	9.4	Mas Control Length (mm)	9.5	
Minimum Concentration to Inhibit Growth (MCIG)	0.25			mg/ml

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.1	0.1	T-test
LOEL	3	0.25	T-test
LC50	2.116	EC50	0.291
95% CL	1.673 -- 2.677	95% Confidence limits	0.222 ---- 0.381
Test Teratogenic Index (TI = LC50/EC50):		7.28	
95% Confidence limits		5.08 -- 10.42	

Percent effect	LC	EC
5	1.0190192	0.085
16	1.3604334	0.138
50	2.1162473	0.291
84	3.2919677	0.611
95	4.3949151	0.992

Cyclophosphamide w w/o MAS #1

FETAX with METABOLIC ACTIVATION (MA)

Summary Sheet

Test Material: P3M1		Test No. 1
Source:		Investigator: Hull
CAS No.	Lot No.	Lab: Bantle
Composition /Purity:		Start Date:
Solvent		End Date:
Conc.:		Units: ma/ml

CONTROLS

Control	Mortality Record	Malformation Record
FETAX	1 : 80 x 100 = 1 %	7 : 79 x 100 = 9 %
Solvent	_____ : _____ x 100 = _____ %	_____ : _____ x 100 = _____ %
Metabolic Activation (MA)	4 : 40 x 100 = 2.5 %	9 : 36 x 100 = 25 %
MA + Solvent	_____ : _____ x 100 = _____ %	_____ : _____ x 100 = _____ %
Cyclophosphamide (+)	40 : 40 x 100 = 100 %	- : - x 100 = - %
Cyclophosphamide (-)	_____ : _____ x 100 = _____ %	_____ : _____ x 100 = _____ %
CO-MA + test material	8 : 40 x 100 = 20 %	10 : 32 x 100 = 31 %

LENGTH DATA

MA + Solvent Control length	mm
Minimum Concentration to Inhibit Growth (MCIG)	3.0, 0.1 MAS

TEST MATERIAL RESULTS

Test	Mortality		Malformation		Statistical Test Used
	no MAS	MAS	no MAS	MAS	
N ₅₀ EL	7	0.1	NA	NA	
L ₅₀ EL	9	2.0	3	0.05	

LC50 (95% confidence limits)		EC50 (95% confidence limits)	
no MAS	MAS	no MAS	MAS
8.725	1.522	3.728	0.130
7.304 - 10.42	0.937 - 2.473	3.30 - 4.17	0.066 - 0.256

TI - no MAS (95% confidence limits)		TI - MAS (95% confidence limits)	
2.34		11.72	
1.90 - 2.89		5.09 - 26.97	

Cyclophosphamide w/inter MAS # 7
FETAX with METABOLIC ACTIVATION (MA)
Summary Sheet

Test Material: P3M1		Test No. 2
Source:		Investigator: Hull
CAS No.	Lot No.	Lab: Bantle
Composition /Purity:		Start Date:
Solvent		End Date:
Conc.:		Units: mg/ml

CONTROLS

Control	Mortality Record	Malformation Record
FETAX	3 : 80 x 100 = 4 %	8 : 77 x 100 = 10 %
Solvent	: x 100 = %	: x 100 = %
Metabolic Activation (MA)	1 : 40 x 100 = %	5 : 39 x 100 = %
MA + Solvent	: x 100 = %	: x 100 = %
Cyclophosphamide (+)	40 : 40 x 100 = 100 %	- : - x 100 = - %
Cyclophosphamide (-)	: x 100 = %	: x 100 = %
CO-MA + test material	1 : 40 x 100 = %	6 : 39 x 100 = %

LENGTH DATA

MA + Solvent Control length	mm
Minimum Concentration to Inhibit Growth (MCIG)	3.0, 0.05 MAS

TEST MATERIAL RESULTS

Test	Mortality		Malformation		Statistical Test Used
	no MAS	MAS	no MAS	MAS	
N ~~NOEL~~	7	0.75	NA	NA	
L ~~NOEL~~	9	9	3	0.05	

LC50 (95% confidence limits)		EC50 (95% confidence limits)	
no MAS	MAS	no MAS	MAS
8.945	0.741	4.034	0.110
6.254 - 12.79	0.648 - 0.848	3.42 - 4.76	0.072 - 0.168

TI - no MAS (95% confidence limits)		TI - MAS (95% confidence limits)	
2.22		6.77	
1.5 - 3.29		4.33 - 10.57	

Cyclophosphamide w/lot 1 R55-3

FETAX with METABOLIC ACTIVATION (MA)

Summary Sheet

Test No. 4	
Test Material: P3M1	Investigator: Hull
Source:	Lab: Bantle
CAS No.	Lot No.
Composition /Purity:	Start Date:
Solvent	End Date:
Conc.:	Units: mg/ml

CONTROLS

Control	Mortality Record	Malformation Record
FETAX	7 : 80 x 100 = 9 %	7 : 73 x 100 = 9.6 %
Solvent	: x 100 = %	: x 100 = %
Metabolic Activation (MA)	7 : 40 x 100 = %	: 33 x 100 = %
MA + Solvent	: x 100 = %	: x 100 = %
Cyclophosphamide (+)	40 : 40 x 100 = 100 %	- : - x 100 = - %
Cyclophosphamide (-)	: x 100 = %	: x 100 = %
CO-MA + test material	36 : 40 x 100 = %	4 : 4 x 100 = 4 %

LENGTH DATA

MA + Solvent Control length	mm
Minimum Concentration to Inhibit Growth (MCIG)	3.0, 0.05 MAS

TEST MATERIAL RESULTS

Test	Mortality		Malformation		Statistical Test Used
	no MAS	MAS	no MAS	MAS	
N ₀ OEEL	5	0.25	NA	0.1	
L ₀ OEEL	9	9	3	0.25	

LC50 (95% confidence limits)		EC50 (95% confidence limits)	
no MAS	MAS	no MAS	MAS
9.74	1.006	3.958	0.171
3.3 - 28.8	0.1 - 10.12	3.546 - 4417	0.135 - 0.216

TI - no MAS (95% confidence limits)		TI - MAS (95% confidence limits)	
2.46		5.89	
0.83 - 7.31		0.58 - 59.96	

Supplemental: w/o MAS #1

FETAX Summary Sheet

Test No. 202-05

Test Material	P3M1 w/o MAS	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	-	Lot No.	-
Composition/Purity	-	Test Start Date:	18 OCT 94 <i>93</i>
Solvent	-	Test End Date	22 OCT 94 <i>93</i>
Conc.	-	Test Units (i.e., mg/ml)	MG/ML

2
4
93

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	6.8	6.9	6.7	6.6	
Control		6.7	6.7	6.8	6.8
Highest Concentration		7.0	7.0	7.0	6.9

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LIM

Total Number

Mortality Record

Malformation Record

FETAX Control

0 : 80

X 100 =

0%

8 : 80

X 100 =

10.0%

Solvent Control

:

X 100 =

:

X 100 =

Control Length (mm) 9.682

Solvent Control Length (mm) -

Minimum Concentration to Inhibit Growth (MCIG)

3.0

MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	6	4	T-test
LOEL	N.A.	N.A.	T-test
LC50	6.530 ✓	EC50	4.407 ✓
95% CL	6.226 -- 6.849	95% Confidence limits	4.029 ---- 4.820
Test Teratogenic Index (TI = LC50/EC50):		1.48 ✓	
95% Confidence limits		1.34 -- 1.64	

Replacand = MAS # 1

FETAX Summary Sheet

Test No. 202-05

Test Material	P3M1 w/ MAS	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	-	Lot No.	-
Composition/Purity	-	Test Start Date:	18 OCT 94
Solvent	-	Test End Date	22 OCT 94
Conc.	-	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	6.8	6.9	6.7	6.6	
Control		6.7	6.7	6.8	6.8
Highest Concentration		7.2	7.2	7.2	7.2

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LIMITS

Total Number

Mortality Record

Malformation Record

FETAX Control

0 : 80

X 100 =

0%

8 : 80

X 100 =

10.0%

Solvent Control

X 100 =

X 100 =

Control Length (mm)

9.164

Solvent Control Length (mm)

-

Minimum Concentration to Inhibit Growth (MCIG)

0.10

MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	1	1	T-test
LOEL	N.A.	N.A.	T-test
LC50	1.118	EC50	1.038
95% CL	1.066	95% Confidence Limits	0.962 --- 1.121
Test Teratogenic Index (TI = LC50/EC50):			1.08
95% Confidence Limits			0.98 --- 1.18

Higher than those in Test # 202-08 & 202-10

FETAX Summary Sheet

Test No. 202-08

Test Material	P3M1 w/o MAS	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	-	Lot No.	-
Composition/Purity	-	Test Start Date:	25 March 1994
Solvent	-	Test End Date	01 April 1994
Conc.	-	Test Units (i.e., mg/ml)	mg/mL

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.4	7.2	7.2	7.2	
Control		7.5	7.3	7.3	7.3
Highest Concentration		7.4	7.3	7.3	7.4

No. Dead or Malformed

X 100 = %

Total Number

	Mortality Record	Malformation Record
FETAX Control	0 : 80 X 100 = 0%	2 : 80 X 100 = 2.5%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm)	9.567	Solvent Control Length (mm) -
Minimum Concentration to Inhibit Growth (MCIG)	3.0	mg/mL

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	7	4	T-test
LOEL	N.A.	N.A.	T-test
LC50	7.937	EC50 5.135	
95% CL	7.522 -- 8.375	95% Confidence limits 4.804 --- 5.488	
Test Teratogenic Index (TI = LC50/EC50):			1.55
95% Confidence limits			1.42 -- 1.68

FETAX Summary Sheet

Test No. 202-08

Test Material	P3M1 W/MAS	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	-	Lot No.	-
Composition/Purity	-	Test Start Date:	25 MARCH 1994
Solvent	-	Test End Date	01 APRIL 1994
Conc.	-	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.4	7.2	7.2	7.2	
Control		7.5	7.3	7.3	7.3
Highest Concentration		7.3	ND	7.4	7.4

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LIMITS

Total Number

Mortality Record

Malformation Record

FETAX Control

0 : 80

X 100 =

0%

6 : 80

X 100 =

7.5%

Solvent Control

X 100 =

X 100 =

Control Length (mm)

9.375

Solvent Control Length (mm)

-

Minimum Concentration to Inhibit Growth (MCIG)

0.1

MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.25	0.05	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.444 ✓	EC50	0.280 ✓
95% CL	0.391 --	0.503	95% Confidence limits 0.128 ---- 0.613
Test Teratogenic Index (TI = LC50/EC50):			1.58 ✓
95% Confidence limits			0.72 -- 3.50

Cyclophosphamide w/0 MAS = 3

Why is this # higher w/ its start date is earlier than 202-0

FETAX Summary Sheet

Test No. 202-10

Test Material	P3M1 w/o MAS	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	-	Lot No.	-
Composition/Purity	-	Test Start Date:	28 FEB 94
Solvent	-	Test End Date	4 MAR 94
Conc.	-	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.2	7.0	7.0	7.0	
Control		7.4	7.2	7.2	7.2
Highest Concentration		7.3	7.2	7.2	7.2

No. Dead or Malformed

X 100 = %

Total Number

Mortality Record

Malformation Record

FETAX Control

0 : 80

X 100 =

0%

0 : 80

X 100 =

0.0%

Solvent Control

X 100 =

X 100 =

Control Length (mm)

10.783

Solvent Control Length (mm)

-

Minimum Concentration to Inhibit Growth (MCIG)

3.0

MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	6	4	T-test
LOEL	N.A.	N.A.	T-test
LC50	7.706 ✓	EC50	5.453 ✓
95% CL	7.211	8.235	95% Confidence limits 5.019 --- 5.925
Test Teratogenic Index (TI = LC50/EC50):			1.41 ✓
95% Confidence limits			1.27 -- 1.57

Cylophosphamide w/ MAF #3

FETAX Summary Sheet

Test No. 202-10

Test Material	P3M1.w/ MAS	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	-	Lot No.	-
Composition/Purity	-	Test Start Date:	28 FEB 94
Solvent	-	Test End Date	4 MAR 94
Conc.	-	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.2	7.0	7.0	7.0	
Control		7.4	7.2	7.2	7.2
Highest Concentration		7.3	7.2	7.2	7.2

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length (mm) 10.031

Solvent Control Length (mm) -

Minimum Concentration to Inhibit Growth (MCIG)

0.05

MG/ML

Mortality Record

Malformation Record

0 : 80

X 100 =

0%

4 : 80

X 100 =

5.0%

X 100 =

X 100 =

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.25	0.25	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.396 ✓	EC50	0.307 ✓
95% CL	0.340	0.462	95% Confidence limits
			0.196 --- 0.480
Test Teratogenic Index (TI = LC50/EC50):			1.29 ✓
95% Confidence limits			0.80 -- 2.07

FETAX with METABOLIC ACTIVATION (MA)

Cyclophosphamide w/o MAS #1

Summary Sheet

Test material: P3M1	Test No. 1
Source:	Investigator: Gillette
CAS No.	Lab: Carvallo
Lot No.	Start Date: 12/10/93
Composition /Purity:	End Date: 12/14/93
Solvent	Conc.: mg/ml

CONTROLS

Control	Mortality Record	Malformation Record
FETAX	4 : 80 x 100 = 5 %	11 : 76 x 100 = 14 %
Solvent	: x 100 = %	: x 100 = %
Metabolic Activation (MA)	0 : 40 x 100 = 0 %	6 : 40 x 100 = 15 %
MA + Solvent	: x 100 = %	: x 100 = %
Cyclophosphamide (+)	5 : 40 x 100 = 13 %	15 : 35 x 100 = 43 %
Cyclophosphamide (-)	1 : 40 x 100 = 3 %	3 : 39 x 100 = 8 %
CO-MA + test material	1 : 40 x 100 = 3 %	0 : 39 x 100 = 0 %

LENGTH DATA

Control length	7.687 mm
Minimum Concentration to Inhibit Growth (MCIG)	0.05 MAS 3 w/o MAS

TEST MATERIAL RESULTS

Test	Mortality		Malformation		Statistical Test Used
	no MAS	MAS	no MAS	MAS	
LOEL	6	-	4	-	Williams
NOEL	7	-	5	-	Williams

LC50 (95% confidence limits)		EC50 (95% confidence limits)	
no MAS	MAS	no MAS	MAS
7.12		5.15	
6.82 - 7.43		4.81 - 5.51	
TI - no MAS (95% confidence limits)		TI - MAS (95% confidence limits)	
1.383		-	

Cyclophosphamide *12/17/93* *#1*
FETAX with METABOLIC ACTIVATION (MA)

Summary Sheet

Test No. 2

Test Material: P3M1

Investigator: Gillette

Source:

Lab: Cervalis

CAS No.

Lot No.

Start Date: 12/17/93

Composition /Purity:

End Date: 12/21/93

Solvent

Conc.:

Units:

CONTROLS

Control	Mortality Record	Malformation Record
FETAX	3 : 80 x 100 = 4 %	3 : 77 x 100 = 4 %
Solvent	: x 100 = %	: x 100 = %
Metabolic Activation (MA)	3 : 40 x 100 = 8 %	4 : 37 x 100 = 11 %
MA + Solvent	: x 100 = %	: x 100 = %
Cyclophosphamide (+)	40 : 40 x 100 = 100 %	- : - x 100 = - %
Cyclophosphamide (-)	40 : 40 x 100 = 100 %	- : - x 100 = - %
CO-MA + test material	5 : 40 x 100 = 13 %	14 : 35 x 100 = 40 %

LENGTH DATA

~~MA + Solvent~~ Control length	8.495 mm
Minimum Concentration to Inhibit Growth (MCIG)	30

TEST MATERIAL RESULTS

Test	Mortality		Malformation		Statistical Test Used
	no MAS	MAS	no MAS	MAS	
LOEL	6	1	5	0.25	William's
NOEL	7	1.25	6	0.5	William's

LC50 (95% confidence limits)		EC50 (95% confidence limits)	
no MAS	MAS	no MAS	MAS
7.5*	1.24*	5.91*	0.86*
7.26 - 7.74	1.16 - 1.32	5.66 - 6.17	0.76 - 0.97
TI - no MAS (95% confidence limits)		TI - MAS (95% confidence limits)	
1.269		1.442	

* Trimmed Spearman-Kärber

FETAX with METABOLIC ACTIVATION (MA)

Cyclophosphamide + CO-MA #3

Summary Sheet

Test Material: <i>P3M1</i>	Test No. <i>3</i>
Source:	Investigator: <i>Gillette</i>
CAS No.	Lot No.
Composition /Purity:	Lab: <i>Corvallis</i>
Solvent	Start Date: <i>12/19/93</i>
Conc.:	End Date: <i>12/23/93</i>
	Units: <i>mg/ml</i>

CONTROLS

Control	Mortality Record	Malformation Record
FETAX	<i>1 : 80 x 100 = 1 %</i>	<i>29 : 79 x 100 = 37 %</i>
Solvent	<i>: x 100 = %</i>	<i>: x 100 = %</i>
Metabolic Activation (MA)	<i>1 : 40 x 100 = 3 %</i>	<i>7 : 39 x 100 = 18 %</i>
MA + Solvent	<i>: x 100 = %</i>	<i>: x 100 = %</i>
Cyclophosphamide (+)	<i>40 : 40 x 100 = 100 %</i>	<i>- : - x 100 = - %</i>
Cyclophosphamide (-)	<i>40 : 40 x 100 = 100 %</i>	<i>- : - x 100 = - %</i>
CO-MA + test material	<i>13 : 40 x 100 = 33 %</i>	<i>27 : 27 x 100 = 100 %</i>

LENGTH DATA

MA + Solvent Control length	<i>8.543 mm</i>
Minimum Concentration to Inhibit Growth (MCIG)	<i>3 w/o MAS 0.25 w/MAS</i>

TEST MATERIAL RESULTS

Test	Mortality		Malformation		Statistical Test Used
	no MAS	MAS	no MAS	MAS	
LOEL	<i>6</i>	<i>0.75</i>	<i>4</i>	<i>0.75</i>	<i>Williams</i>
NOEL	<i>7</i>	<i>1.0</i>	<i>5</i>	<i>1.0</i>	<i>Williams</i>

LC50 (95% confidence limits)		EC50 (95% confidence limits)	
no MAS	MAS	no MAS	MAS
<i>7.36</i>	<i>1.33</i>	<i>3.74</i>	<i>0.36</i>
<i>7.13-7.61</i>	<i>1.24-1.42</i>	<i>1.89-7.42</i>	<i>0.82-0.91</i>
TI - no MAS (95% confidence limits)		TI - MAS (95% confidence limits)	
<i>1.968</i>		<i>1.547 -</i>	

*Cyclophosphamide w/o MAS #4⁷⁸
w MAS*

FETAX with METABOLIC ACTIVATION (MA)
Summary Sheet

Test No. 1

Test Material	UNKNOWN P3M1	Investigator	TURLEY
Source	CSU	Lab	UMD-WREC
CAS No.		Test Start Date	10/13/93
Composition/Purity		Test End Date	10/17/93
Solvent		Test Units (i.e., mg/ml)	mg/L

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	3.21	3.33	3.19	3.27	—
Control	7.75	7.69	7.82	7.77	—
Highest Conc.	—	—	—	—	—

FETAX CONTROL

MORTALITY RECORD

MALFORMATION RECORD

No. Dead or Malformed X 100 = %
Total Number

Solvent Control	1 : 80 X 100 = 1.25%	5 : 79 X 100 = 6.3%
Metabolic Activation Control MAS	0 : 40 X 100 = 0%	4 : 40 X 100 = 10%
Metabolic Activation System plus Solvent Control	— : — X 100 = —%	— : — X 100 = —%
Cyclophosphamide Control (4.0 mg/ml) CP(+)	32 : 40 X 100 = 80%	8 : 8 X 100 = 100%
CO-Metabolic Activation System plus Test Material P3M1 2.0(-)	2 : 40 X 100 = 5%	4 : 38 X 100 = 10.5%
MA + Solvent Control Length	9.78 mm	
Minimum Concentration to Inhibit Growth (MCIG)	MAS - 0.75 NO MAS - 4.0	

TEST MATERIAL RESULTS

TEST	MORTALITY		MALFORMATION	STATISTICAL TEST USED	
NOEL	FAIL	HOMOGENEITY	FAIL	NORMALITY	CHI-SQUARE, BARTLETT'S
LOEL	FAIL	HOMOGENEITY	FAIL	HOMOGENEITY	CHI-SQUARE, BARTLETT'S
LC ₅₀	MAS 2.25	NO MAS 6.72	EC ₅₀	MAS 1.51 (1.22-1.78)	NO MAS 5.30
95% Confidence limits	MAS (1.72-2.93)	NO MAS (6.42-7.04)	95% Confidence Limits	MAS (1.22-1.78)	NO MAS 4.22-5.22
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)				MAS 1.49	NO MAS 1.27

Cyclophosphamide w/MAS #2
wo/MAS

FETAX with METABOLIC ACTIVATION (MA) Summary Sheet

Test No. 2

Test Material <u>UNKNOWN P3M1</u>	Investigator <u>TURNER</u>
Source <u>0.5.0</u>	Lab <u>UMD-WREC</u>
CAS No.	Lot No.
Composition/Purity	Test Start Date <u>10/27/93</u>
Solvent	Test End Date <u>10/31/93</u>
Conc.	Test Units (i.e., mg/ml) <u>mg/L</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	3.20	3.26	3.33	3.21	-
Control	7.84	7.79	7.77	7.81	-
Highest Conc.	-	-	-	-	-

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %		
Solvent Control	1 : 80 X 100 = 1.25 %	6 : 79 X 100 = 7.6 %
Metabolic Activation Control	0 : 40 X 100 = 0 %	4 : 40 X 100 = 10 %
Metabolic Activation System plus Solvent Control	_____ X 100 = _____ %	_____ X 100 = _____ %
Cyclophosphamide Control (4.0 mg/ml)	22 : 40 X 100 = 55 %	11 : 18 X 100 = 61.1 %
CO-Metabolic Activation System plus Test Material	0 : 40 X 100 = 0 %	8 : 40 X 100 = 20 %
MA + Solvent Control Length	mm <u>MAC</u>	
Minimum Concentration to Inhibit Growth (MIC)	<u>1.0</u> <u>4.0</u>	

TEST MATERIAL RESULTS -

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	MAS FAIL HOMO.	NO MAS FAIL HOMO.	Chi-Square / BARTLETT'S
LOEL	MAS FAIL HOMO.	NO MAS FAIL HOMO.	Chi-Square / BARTLETT'S
LC ₅₀	MAS 2.51	NO MAS 6.32	EC ₅₀ 1.22
95% Confidence limits	MAS (2.06-3.05)	NO MAS (5.94-6.72)	95% Confidence Limits MAS (0.29-1.69) NO MAS (4.13-5.69)
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			
MAS 2.06 NO MAS 1.30			

Trimmed Spearman-Kärber

Cyclophosphamide w/ MAS #3 OK
w/ MAS

FETAX with METABOLIC ACTIVATION (MA) Summary Sheet

Test No. 3

Test Material	UNKNOWN P3M1	Investigator	TURLEY
Source	OSU	Lab	UMD-WREC
CAS No.	Lot No.	Test Start Date	11/1/93
Composition/Purity		Test End Date	11/5/93
Solvent	Conc.	Test Units (i.e., mg/ml)	ml/L

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	3.11	3.23	3.09	3.17	—
Control	787	779	790	781	—
Highest Conc.	—	—	—	—	—

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %		
Solvent Control	2 : 80 X 100 = 25 %	5 : 78 X 100 = 6.4 %
Metabolic Activation Control MAS	0 : 40 X 100 = 0 %	3 : 40 X 100 = 7.5 %
Metabolic Activation System plus Solvent Control		
Cyclophosphamide Control (4.0 mg/ml) CP(+)	35 : 40 X 100 = 87.5 %	5 : 5 X 100 = 100 %
CO-Metabolic Activation System plus Test Material P3M1 2.0(-)	0 : 40 X 100 = 0 %	3 : 40 X 100 = 7.5 %
MA + Solvent Control Length	9.85 mm	
Minimum Concentration to Inhibit Growth (MCIG)	MAS-0.75	No MAS-5.0

TEST MATERIAL RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	FAIL HOMOGENEITY	FAIL HOMOGENEITY	Chi-Square, Bartlett's
LOEL	FAIL HOMOGENEITY	FAIL HOMOGENEITY	Chi-Square, Bartlett's
LC ₅₀	MAS 1.76	NO MAS 5.81	EC ₅₀ 1.11
95% Confidence limits	MAS (1.57-1.97)	NO MAS (5.53-6.11)	95% Confidence Limits MAS (0.37-1.41) NO MAS (4.03-5.02)
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)		MAS 1.59	NO MAS 1.29

Cyclophosphamide w MAS #1
 FETAX with METABOLIC ACTIVATION (MA)

Summary Sheet

		Test No.	1
Test Material: P3M1		Investigator: Dawson	
Source: OSU		Lab: Asnland	
CAS No.	Lot No.	Start Date: 6-15-94	
Composition /Purity:		End Date: 6-19-94	
Solvent	Conc.:	Units: mg/ml	

CONTROLS

Control	Mortality Record	Malformation Record
FETAX	1 : 80 x 100 = 1 %	6 : 79 x 100 = 7.6 %
Solvent	: x 100 = %	: x 100 = %
Metabolic Activation (MA)	0 : 40 x 100 = 0 %	3 : 40 x 100 = 7.5 %
MA + Solvent	: x 100 = %	: x 100 = %
Cyclophosphamide (+)	40 : 40 x 100 = 100 %	- : - x 100 = - %
Cyclophosphamide (-)	0 : 40 x 100 = 0 %	3 : 40 x 100 = 7.5 %
CO-MA + test material	0 : 40 x 100 = 0 %	4 : 40 x 100 = 10 %

LENGTH DATA

MA + Solvent Control length	mm
Minimum Concentration to Inhibit Growth (MCIG)	

TEST MATERIAL RESULTS

Test	Mortality		Malformation		Statistical Test Used
	no MAS	MAS	no MAS	MAS	
LOEL	5	0.5	3	0.1	
NOEL	9	6	4	0.25	

LC50 (95% confidence limits)		EC50 (95% confidence limits)	
no MAS	MAS	no MAS	MAS
6.218	1.694	4.879	0.815
5.843 - 6.618	1.441 - 1.992	4.462 - 5.336	0.608 - 1.093

TI - no MAS (95% confidence limits)		TI - MAS (95% confidence limits)	
1.27		2.08	
1.14 - 1.42		1.49 - 2.91	

Cyclophosphamide w/ MAS # =
FET (with METABOLIC ACTIVATION) (MA)
Summary Sheet

Test No. 2			
Test Material: P3M1		Investigator: Dawson	
Source:		Lab: Ashland	
CAS No.	Lot No.	Start Date: 6-22-94	
Composition /Purity:		End Date: 6-26-94	
Solvent	Conc.:	Units: mg/ml	
-pH-	Day 0	Day 1	Day 3
Stock			
Control	X		
Highest Conc.	X		

CONTROLS

Control	Mortality Record	Malformation Record
FETAX	$\frac{0}{80} \times 100 = 0\%$	$\frac{3}{80} \times 100 = 3.8\%$
Solvent	$\frac{\quad}{\quad} \times 100 = \quad\%$	$\frac{\quad}{\quad} \times 100 = \quad\%$
Metabolic Activation (MA)	$\frac{1}{40} \times 100 = 2.5\%$	$\frac{1}{39} \times 100 = 2.6\%$
MA + Solvent	$\frac{\quad}{\quad} \times 100 = \quad\%$	$\frac{\quad}{\quad} \times 100 = \quad\%$
Cyclophosphamide (+)	$\frac{40}{40} \times 100 = 100\%$	$\frac{-}{-} \times 100 = -\%$
Cyclophosphamide (-)	$\frac{0}{40} \times 100 = 0\%$	$\frac{2}{40} \times 100 = 5\%$
CO-MA + test material	$\frac{0}{40} \times 100 = 0\%$	$\frac{1}{40} \times 100 = 2.5\%$

LENGTH DATA

MA + Solvent Control length	mm
Minimum Concentration to Inhibit Growth (MCIG)	

TEST MATERIAL RESULTS

Test	Mortality		Malformation		Statistical Test Used
	no MAS	MAS	no MAS	MAS	
NOEL	5	1	3	0.1	T-test
LOEL	6	6	4	0.25	T-test

LC50 (95% confidence limits)		EC50 (95% confidence limits)	
no MAS	MAS	no MAS	MAS
6.122	1.229	4.864	0.527
5.737 - 6.532	0.356 - 4.245	4.445 - 5.323	0.389 - 0.716
TI - no MAS (95% confidence limits)		TI - MAS (95% confidence limits)	
1.26		2.33	
1.13 - 1.41		0.65 - 8.85	

Cyclophosphamide w/ MAS #3

FETAX Summary Sheet

Test No. 3

Test Material	P3M1	Investigator	Dawson
Source		Laboratory	Ashland
CAS No.	Lot No.	Test Start Date:	7/18/94
Composition/Purity		Test End Date	7/22/94
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.1	7.07	7.07	7.1	
Control		7.59	7.42	7.4	7.24
Highest Concentration		7.24/7.24	7/6.99	7.01/7	--/6.89

No. Dead or Malformed		
X 100 = %		
Total Number		
	Mortality Record	Malformation Record
FETAX Control	0 : 80 X 100 = 0%	2 : 80 X 100 = 2.5%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm)	Solvent Control Length (mm)	
Minimum Concentration to Inhibit Growth (MCIG)	mg/ml	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	5	4	T-test
LOEL	66	5	T-test
LC50	6.474	EC50	5.014
95% CL	5.960 -- 7.034	95% Confidence limits	4.668 ---- 5.386
Test Teratogenic Index (TI = LC50/EC50):		1.29	
95% Confidence limits		1.16 -- 1.44	

● *Cyclophosphamide* ● *W MP = 3*

FETAX Summary Sheet

		Test No.	3
Test Material	P3M1-MAS	Investigator	Dawson
Source	-	Laboratory	Ashland
CAS No.	Lot No.	Test Start Date:	7/18/94
Composition/Purity		Test End Date	7/22/94
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7.1	7.07	7.07	7.1	
Control		7.59	7.42	7.4	7.24
Highest Concentration		7.24/7.24	7/6.99	7.01/7	--/6.89

No. Dead or Malformed			
X 100 = %			
Total Number		Mortality Record	Malformation Record
FETAX Control	0 : 80	X 100 = 0%	2 : 80 X 100 = 2.5%
Solvent Control	:	X 100 =	: X 100 =
Control Length (mm)	Solvent Control Length (mm)		
Minimum Concentration to Inhibit Growth (MCIG)			mg/ml

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	1	0.05	T-test
LOEL	1.25	0.5	T-test
LC50	1.040	EC50	0.562
95% CL	0.543 -- 1.995	95% Confidence limits	0.562 ---- 0.562
Test Teratogenic Index (TI = LC50/EC50):		1.85	
95% Confidence limits		0.97 --	3.55

Cyclophosphamide w/o MAS #1
w MAS #1

✓ DK

FETAX SUMMARY SHEET

Test Material P3M I		Test No. 1
Source TLS Miosome Lot 4		Investigator Fort
CAS No.	Lot No.	Lab SBL
Composition/Purity		Test Start Date 9/20/93
Solvent		Test End Date 9/24/93
Conc.		Test Units (i.e., mg/ml) mg/mL

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.2	7.2	7.2	7.3	
Control	8.0	8.0	7.9	7.9	
Highest Conc.	7.0	7.1	7.2	7.1	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %	0 : 80 X 100 = 0 %	6 : 80 X 100 = 7.5 %
Solvent Control MAS	1 : 40 X 100 = 2.5 %	3 : 39 X 100 = %
Control Length 91.4 mm	Solvent Control Length 90.2 mm	
Minimum Concentration to Inhibit Growth (MCIG) unactivated 4.0 activated 0.05		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	—	—	NA
LOEL	—	—	NA
LC ₅₀ Activated 1.12 Unactivated 5.74	EC ₅₀ Activated 0.32 Unactivated 5.19		
95% Confidence limits 0.78 - 1.23 / 5.23 - 6.09 95% Confidence Limits 0.26 - 0.40 / 4.38 - 5.39			
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀) 3.5 ACT / 1.1 UAT			

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 = _____ %

Cyclophosphamide WOMAS
WOMAS #7

FETAX SUMMARY SHEET

Test No. 2

Test Material	P3M1	Investigator	Fort
Source	TLS	Lab	SBI
CAS No.		Lot No.	
Composition/Purity		Test Start Date	10/18/93
Solvent		Test End Date	10/22/93
Coac.		Test Units (i.e., mg/ml)	mg/mL

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.0	7.1	7.0	7.0	
Control	7.9	8.0	8.0	8.0	
Highest Coac.	7.1	7.0	7.1	7.2	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %		
Stock Control	4 : 80 X 100 = 5 %	6 : 76 X 100 = 6.6 %
Control Length 0.95 mm	3 : 40 X 100 = 7.5 %	3 : 37 X 100 = 8.1 %
Minimum Concentration to Inhibit Growth (MCIG)	Solvent Control Length 0.76 mm	
	4.0 / 1.25 ACT	

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	3.0 / 0.05	3.0 / 0.1	Williams
LOEL	4.0 / 0.1	4.0 / 0.25	Williams
LC ₅₀	7.35 ^{UACT} / 1.62 ^{ACT}	EC ₅₀ 5.34 ^{UACT} / 0.53 ^{ACT}	
95% Confidence Limits 6.95-7.87 / 1.48-1.74		95% Confidence Limits 4.92-5.82 / 0.49-0.58	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 1.38^{UACT} / 3.06^{ACT}

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 = ____ %

Cyclophosphamide WORMS #3
WORMS #3

FETAX SUMMARY SHEET

Test No. 3

Test Material	P3M1	Investigator	Fort
Source	ILS	Lab	SBL
CAS No.		Lot No.	
Composition/Purity		Test Start Date	10/20/93
Solvent		Test End Date	10/24/93
Conc.		Test Units (i.e., mg/ml)	mg/ml

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.0	7.1	7.0	7.1	
Control	7.9	7.8	7.9	7.8	
Highest Conc.	7.0	7.0	7.0	7.1	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %		
	4 : 80 x 100 = 5%	5 : 76 x 100 = 6.6%
Solvent Control	2 : 40 x 100 = 5%	3 : 38 x 100 = 7.9%
Control Length 0.92 mm	Solvent Control Length 0.83 mm	
Minimum Concentration to Inhibit Growth (MCIG)	6.0 μ ACT	0.05 ACT

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	6.0 / 0.5	- / -	Williams
LOEL	7.0 / 0.75	3.0 / 0.05	Williams
LC ₅₀	7.92 μ ACT / 1.38 ACT	EC ₅₀ 5.05 μ ACT / 0.48 ACT	
95% Confidence limits	7.85-8.10 / 1.32-1.44	95% Confidence Limits	4.95-5.17 / 0.42-0.54
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			
1.56 μ ACT / 2.88 ACT			

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 = ____ %

CAFFEINE
DATA SUMMARY SHEETS
PHASE III-PART 2

Caffeine

James Rayburn

Caffeine

James Rayburn

Data Sheets Follow

Coffine w/0.1M #5

FETAX Summary Sheet

Test No. 2

Test Material	P3M2 unacivated	Investigator	James Rayburn
Source	-	Laboratory	Bantle
CAS No.	Lot No.	Test Start Date:	July 26, 1994
Composition/Purity		Test End Date	July 30, 1994
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7	6.9	6.8	6.8	
Control		7.9	7.5	7.5	7.2
Highest Concentration		6.2	6.3	6.4	6.4

No. Dead or Malformed		
X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	4 : 80 X 100 = 5%	5 : 76 X 100 = 6.6%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm)	9.16	Solvent Control Length (mm)
Minimum Concentration to Inhibit Growth (MCIG)	0.1	mg/ml

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.25	0.1	T-test
LOEL	0.3	0.125	T-test
LC50	0.462	EC50	0.139
95% CL	0.411 -- 0.519	95% Confidence limits	0.128 ---- 0.151
Test Teratogenic Index (TI = LC50/EC50):		3.32	
95% Confidence limits		2.88 --	3.83

Percent effect	LC	EC
5	0.2912074	0.095
16	0.3495876	0.111
50	0.4622664	0.139
84	0.6112638	0.175
95	0.7338077	0.203

FETAX Summary Sheet

Confidence in FETAX #2

Test Material		P3M2 activated	Investigator	James Rayburn
Source			Laboratory	Bantle
CAS No.	Lot No.		Test Start Date:	July 26, 1994
Composition/Purity			Test End Date	July 30, 1994
Solvent	Conc.		Test Units (i.e., mg/ml)	mg/ml

		Day 0	Day 1	Day 2	Day 3	Day 4
pH	Stock	6.8	7	6.8	6.8	
	Control		7.7	7.6	7.5	7.2
	Highest Concentration		6	6.3	6.4	6.4

No. Dead or Malformed		Mortality Record		Malformation Record	
X 100 = %	FETAX Con	4 : 80	X 100 = 5%	5 : 76	X 100 = 6.6%
Total Number	MAS control	1 : 40	X 100 = 2.5%	2 : 39	X 100 = 5.1%
CP 4 mg/l	Postive CP	40 : 40	X100 = 100.0%	:	X 100 =
CP 4 mg/l	Negative CP	3 : 40	X 100 = 7.5%	22 : 37	X 100 = 59.5%
Control Length (mm)		9.16	Mas Control Length (mm)		9.5
Minimum Concentration to Inhibit Growth (MCIG)				0.1	mg/ml

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used		
NOEL	0.18	N.A.	T-test		
LOEL	0.28	0.1	T-test		
LC50	0.222	EC50	0.088		
95% CL	0.200	--	0.246	95% Confidence limits	0.074 ---- 0.104
Test Teratogenic Index (TI = LC50/EC50):				2.54	
95% Confidence limits				2.07	-- 3.11

Percent effect	LC	EC
5	0.1429636	0.052
16	0.1701761	0.064
50	0.2221301	0.088
84	0.2899453	0.121
95	0.3451352	0.149

Coffin 450000 #3

FETAX Summary Sheet

Test Material- P3M2 unactivated		Investigator	James Rayburn
Source -		Laboratory	Bantle
CAS No.	Lot No.	Test Start Date:	July 26, 1994
Composition/Purity		Test End Date	July 30, 1994
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7	6.9	6.8	6.8	
Control		7.9	7.5	7.5	7.2
Highest Concentration		6.2	6.3	6.4	6.4

No. Dead or Malformed $\times 100 = \%$ Total Number	Mortality Record		Malformation Record	
	2 : 80	$\times 100 = 3\%$	4 : 78	$\times 100 = 5.1\%$
	: $\times 100 =$		: $\times 100 =$	
	: $\times 100 =$		: $\times 100 =$	
FETAX Control				
Solvent Control				
Control Length (mm) 9.84		Solvent Control Length (mm)		
Minimum Concentration to Inhibit Growth (MCIG)		0.1 mg/ml		

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.3	0.1	T-test
LOEL	0.5	0.125	T-test
LC50	0.551	EC50	0.129
95% CL	0.455 -- 0.667	95% Confidence limits	0.120 ---- 0.140
Test Teratogenic Index (TI = LC50/EC50):			4.27
95% Confidence limits			3.50 -- 5.20

Colony w/ MAS #3

FETAX Summary Sheet

Test No. 3

Test Material	P3M2 activated	Investigator	James Rayburn
Source	-	Laboratory	Bantle
CAS No.	Lot No.	Test Start Date:	July 26, 1994
Composition/Purity		Test End Date	July 30, 1994
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	6.8	7	6.8	6.8	
Control		7.7	7.6	7.5	7.2
Highest Concentration		6	6.3	6.4	6.4

No. Dead or Malformed	Mortality Record		Malformation Record	
X 100 = % FETAX Con	2 : 80	X 100 = 3%	4 : 78	X 100 = 5.1%
Total Number MAS control	1 : 40	X 100 = 2.5%	2 : 39	X 100 = 5.1%
CP 4 mg/l Postive CP	20 : 20	X100 = 100.0%		X 100 =
CP 4 mg/l Negative CP	5 : 20	X 100 = 25.0%	5 : 15	X 100 = 33.3%
Control Length (mm)	9.84	Mas Control Length (mm)	9.6	
Minimum Concentration to Inhibit Growth (MCIG)		0.1	mg/ml	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.1	N.A.	T-test
LOEL	0.12	0.1	T-test
LC50	0.213	EC50	0.110
95% CL	0.120 -- 0.380	95% Confidence limits	0.100 ---- 0.115
Test Teratogenic Index (TI = LC50/EC50):		1.94	
95% Confidence limits		1.10 --	3.50

Coffine - 0.0005 #1

FETAX with METABOLIC ACTIVATION (MA)

Summary Sheet

Test Material: <u>P3M2</u>		Test No. <u>1</u>
Source:		Investigator: <u>Hull</u>
CAS No.	Lot No.	Lab: <u>Bantle</u>
Composition /Purity:		Start Date: <u>12-8-93</u>
Solvent		End Date: <u>12-12-93</u>
Conc.:		Units: <u>mg/ml</u>

CONTROLS

Control	Mortality Record	Malformation Record
FETAX	<u>4 : 80</u> x 100 = <u>5</u> %	<u>7 : 76</u> x 100 = <u>9.2</u> %
Solvent	_____ : _____ x 100 = _____ %	_____ : _____ x 100 = _____ %
Metabolic Activation (MA)	<u>1 : 40</u> x 100 = _____ %	<u>3 : 39</u> x 100 = _____ %
MA + Solvent	_____ : _____ x 100 = _____ %	_____ : _____ x 100 = _____ %
Cyclophosphamide (+)	<u>40 : 40</u> x 100 = <u>100</u> %	<u>+</u> : <u>-</u> x 100 = <u>-</u> %
Cyclophosphamide (-)	_____ : _____ x 100 = _____ %	_____ : _____ x 100 = _____ %
CO-MA + test material	_____ : _____ x 100 = _____ %	_____ : _____ x 100 = _____ %

LENGTH DATA

MA + Solvent Control length	mm
Minimum Concentration to Inhibit Growth (MCIG)	<u>0.1, 0.1 w/MAS</u>

TEST MATERIAL RESULTS

Test	Mortality		Malformation		Statistical Test Used
	no MAS	MAS	no MAS	MAS	
<u>N</u> <u>8</u> <u>OE</u> <u>L</u>	<u>0.5</u>	<u>0.2</u>	<u>NA</u>	<u>NA</u>	
<u>L</u> <u>8</u> <u>OE</u> <u>L</u>	<u>0.6</u>	<u>0.6</u>	<u>0.1</u>	<u>0.1</u>	

LC50 (95% confidence limits)		EC50 (95% confidence limits)	
no MAS	MAS	no MAS	MAS
<u>0.785</u>	<u>0.277</u>	<u>0.094</u>	<u>0.119</u>
<u>0.531 - 1.116</u>	<u>0.251 - 0.305</u>	<u>0.063 - 0.142</u>	<u>0.107 - 0.132</u>

TI - no MAS (95% confidence limits)	TI - MAS (95% confidence limits)
<u>8.32</u>	<u>2.33</u>
<u>4.72 - 14.66</u>	<u>2.02 - 2.69</u>

Refers to: MA #2

Summary Sheet

Summary Sheet		Test No.
Test Material:	P3M2	3
Source:		Investigator: Hull
CAS No.	Lot No.	Lab:
Composition /Purity:		Start Date:
Solvent	Conc.:	End Date:
		Units:

CONTROLS

CONTROLS		
Control	Mortality Record	Malformation Record
FETAX - AB	6 : 80 x 100 = 8 %	6 : 74 x 100 = 8.1 %
Solvent	_____ : _____ x 100 = _____ %	_____ : _____ x 100 = _____ %
Metabolic Activation (MA)	_____ : _____ x 100 = _____ %	_____ : _____ x 100 = _____ %
MA + Solvent	2 : 40 x 100 = 5 %	8 : 38 x 100 = 21 %
Cyclophosphamide (+)	40 : 40 x 100 = 100 %	- : - x 100 = - %
Cyclophosphamide (-)	10 : 40 x 100 = 25 %	12 : 30 x 100 = 40 %
CO-MA + test material	_____ : _____ x 100 = _____ %	_____ : _____ x 100 = _____ %

LENGTH DATA

MA + Solvent Control length	mm
Minimum Concentration to Inhibit Growth (MCIG)	0.1, 0.1 MAS

TEST MATERIAL RESULTS

Test	Mortality		Malformation		Statistical Test Used
	no MAS	MAS	no MAS	MAS	
POEL	0.6	NA	NA	NA	
LOEL	0.5	0.8	0.1	0.1	

LC50 (95% confidence limits)		EC50 (95% confidence limits)	
no MAS	MAS	no MAS	MAS
0.846	0.282	0.103	0.101
0.692 - 1.036	0.250 - 0.318	0.089 - 0.120	0.088 - 0.116

TI - no MAS (95% confidence limits)	TI - MAS (95% confidence limits)
6.20	2.80
(6.39 - 10.52)	2.33 - 3.36

Compare w w/ MA #3
FETAX with METABOLIC ACTIVATION (MA)
Summary Sheet

Test Material: <u>PBM2</u>		Test No. <u>4</u>
Source:		Investigator: <u>Hill</u>
CAS No.	Lot No.	Lab: <u>Bantle</u>
Composition /Purity:		Start Date: <u>2/2/94</u>
Solvent		End Date: <u>2/6/94</u>
Conc.:		Units: <u>mg/mL</u>

CONTROLS

Control	Mortality Record	Malformation Record
FETAX	<u>0 : 80</u> x 100 = <u>0</u> %	<u>8 : 80</u> x 100 = <u>10</u> %
Solvent	_____ x 100 = _____ %	_____ x 100 = _____ %
Metabolic Activation (MA)	<u>1 : 40</u> x 100 = <u>3</u> %	<u>10 : 39</u> x 100 = <u>26</u> %
MA + Solvent	_____ x 100 = _____ %	_____ x 100 = _____ %
Cyclophosphamide (+)	<u>40 : 40</u> x 100 = <u>100</u> %	<u>- : -</u> x 100 = <u>-</u> %
Cyclophosphamide (-)	<u>39 : 40</u> x 100 = <u>98</u> %	<u>1 : 1</u> x 100 = <u>100</u> %
CO-MA + test material	_____ x 100 = _____ %	_____ x 100 = _____ %

LENGTH DATA

MA + Solvent Control length	mm
Minimum Concentration to Inhibit Growth (MCIG)	<u>0.15</u> , <u>0.1 MAS</u>

TEST MATERIAL RESULTS

Test	Mortality		Malformation		Statistical Test Used
	no MAS	MAS	no MAS	MAS	
<u>NOEL</u>	<u>0.6</u>	<u>NA</u>	<u>0.1</u>	<u>NA</u>	
<u>LOEL</u>	<u>0.8</u>	<u>0.3</u>	<u>0.125</u>	<u>0.1</u>	

LC50 (95% confidence limits)		EC50 (95% confidence limits)	
no MAS	MAS	no MAS	MAS
<u>1.043</u>	<u>0.321</u>	<u>0.129</u>	<u>0.115</u>
<u>0.680 - 1.599</u>	<u>0.256 - 0.403</u>	<u>0.114 - 0.144</u>	<u>0.098 - 0.135</u>

TI - no MAS (95% confidence limits)		TI - MAS (95% confidence limits)	
<u>5.11</u>		<u>2.79</u>	
<u>5.20 - 12.63</u>		<u>2.11 - 3.68</u>	

Colony count = 1

FETAX Summary Sheet

Test No. 205-01

Test Material	P3M2 w/o MAS	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	- Lot No. -	Test Start Date:	24 JAN 94
Composition/Purity	-	Test End Date	28 JAN 94
Solvent	- Conc -	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7.4	7.3	7.2	7.2	
Control		7.2	7.1	7.1	7.1
Highest Concentration		7.5	7.5	7.4	7.4

No. Dead or Malformed		
X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	1 : 80 X 100 = 1%	5 : 79 X 100 = 6.3%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm)	9.995	Solvent Control Length (mm) -
Minimum Concentration to Inhibit Growth (MCIG)	0.10	MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.25	0.1	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.553	EC50	0.137
95% CL	0.417 -- 0.733	95% Confidence limits	0.127 ---- 0.149
Test Teratogenic Index (TI = LC50/EC50):		4.02	
95% Confidence limits		3.00 -- 5.40	

Offspring AIMS #1

FETAX Summary Sheet

Test No. 205-01

Test Material	P3M2 w/ MAS	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	-	Lot No.	-
Composition/Purity	-	Test Start Date:	24 JAN 94
Solvent	-	Test End Date	28 JAN 94
Conc.	-	Test Units (i.e., mg/ml)	MG/ML

		Day 0	Day 1	Day 2	Day 3	Day 4
pH	Stock	7.4	7.3	7.2	7.2	
	Control		7.2	7.1	7.1	7.1
	Highest Concentration		7.5	7.6	7.4	7.4

No. Dead or Malformed		
X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	2 : 80 X 100 = 3%	4 : 78 X 100 = 5.1%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm)	9.538	Solvent Control Length (mm) -
Minimum Concentration to Inhibit Growth (MCIG)	0.10	MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.22	N.A.	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.240	EC50	0.097
95% CL	0.221 -- 0.262	95% Confidence limits	0.069 ---- 0.135
Test Teratogenic Index (TI = LC50/EC50):		2.49	
95% Confidence limits		1.76 -- 3.53	

Revised 7/10/95 #2

FETAX Summary Sheet

Test No. 205-02

Test Material	P3M2 w/o MAS	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	-	Lot No.	-
Test Start Date:	31 JAN 94	Test End Date	4 FEB 94
Composition/Purity	-	Test Units (i.e., mg/ml)	MG/ML
Solvent	-	Conc.	-

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7.4	7.4	7.3	7.3	
pH Control		7.3	7.2	7.4	7.2
pH Highest Concentration		7.6	7.4	7.4	7.4

No. Dead or Malformed		
X 100 = %		
Total Number		
	Mortality Record	Malformation Record
FETAX Control	3 : 80 X 100 = 4%	4 : 77 X 100 = 5.2%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm)	9.65	Solvent Control Length (mm) -
Minimum Concentration to Inhibit Growth (MCIG)	0.10	MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.3	N.A.	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.567	EC50	0.129
95% CL	0.383 -- 0.839	95% Confidence limits	0.118 ---- *****
Test Teratogenic Index (TI = LC50/EC50):		4.38	
95% Confidence limits		2.92 -- 6.55	

Supplemental WMS# 2

FETAX Summary Sheet

Test No. 205-02

Test Material	P3M2 w/ MAS	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	Lot No.	Test Start Date:	31 JAN 94
Composition/Purity	-	Test End Date	4 FEB 94
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7.4	7.4	7.3	7.3	
Control		7.3	7.2	7.4	7.2
Highest Concentration		7.6	7.5	--	--

No. Dead or Malformed	MALFORMATION EXCEED ASTM LIMITS			
X 100 = %				
Total Number	Mortality Record		Malformation Record	
FETAX Control	1 : 80	X 100 = 1%	9 : 79	X 100 = 11.4%
Solvent Control	:	X 100 =	:	X 100 =
Control Length (mm)	9.619	Solvent Control Length (mm)	-	
Minimum Concentration to Inhibit Growth (MCIG)	0.10		MG/ML	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.2	N.A.	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.226	EC50	0.104
95% CL	0.217	95% Confidence limits	0.095 --- 0.114
Test Teratogenic Index (TI = LC50/EC50):		2.17	
95% Confidence limits		1.97 --- 2.39	

Capitex 5/21/95 #3

FETAX Summary Sheet

Test No. 205-03

Test Material	P3M2 w/o MAS	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	-	Lot No.	-
Composition/Purity	-	Test Start Date:	28 FEB 94
Solvent	-	Test End Date	4 MAR 94
Conc.	-	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7.5	7.2	7.3	7.3	
Control		7.4	7.2	7.2	7.2
Highest Concentration		7.8	7.4	7.4	7.4

No. Dead or Malformed		
X 100 = %		
Total Number		
FETAX Control	Mortality Record	Malformation Record
Solvent Control		
Control Length (mm)	10.796	Solvent Control Length (mm) -
Minimum Concentration to Inhibit Growth (MCIG)	0.10	MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.4	N.A.	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.711	EC50	0.114
95% CL	0.570	95% Confidence limits	0.097 --- 0.134
Test Teratogenic Index (TI = LC50/EC50):		6.22	
95% Confidence limits		4.73 --- 8.17	

Caffeine w/ MAS #3

FETAX Summary Sheet

Test No. 205-03

Test Material	P3M2 w/ MAS	Investigator	FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	Lot No.	Test Start Date:	28 FEB 94
Composition/Purity	-	Test End Date	4 MAR 94
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7.5	7.2	7.3	7.3	
Control		7.4	7.2	7.2	7.2
Highest Concentration		7.4	7.2	---	---

No. Dead or Malformed	MALFORMATION EXCEED ASTM LIMITS			
X 100 = %				
Total Number	Mortality Record		Malformation Record	
FETAX Control	0 : 80	X 100 = 0%	6 : 80	X 100 = 7.5%
Solvent Control	:	X 100 =	:	X 100 =
Control Length (mm)	9.787	Solvent Control Length (mm)	-	
Minimum Concentration to Inhibit Growth (MCIG)	0.10	MG/ML		

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.22	N.A.	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.290	EC50	0.098
95% CL	0.249	95% Confidence limits	0.080 --- 0.118
Test Teratogenic Index (TI = LC50/EC50):		2.97	
95% Confidence limits		2.32 --- 3.80	

Caffeine w MAS #1
w/o MAS

FETAX with METABOLIC ACTIVATION (MA)
Summary Sheet

Test No. 1

Test Material	UNKNOWN P3M2	Investigator	TURLEY
Source	0.50	Lab	UMD WREC
CAS No.		Lot No.	
Composition/Purity		Test Start Date	11/3/94
Solvent		Conc.	
		Test End Date	1/7/94
		Test Units (i.e., mg/ml)	mg/ml

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.42	7.35	7.40	7.38	—
Control	7.80	7.77	7.79	7.75	—
Highest Conc.	—	—	—	—	—

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %	1 : 40 X 100 = 2.5 %	2 : 39 X 100 = 5.1 %
Solvent Control	— : — X 100 = — %	— : — X 100 = — %
Metabolic Activation Control	1 : 40 X 100 = 2.5 %	2 : 39 X 100 = 5.1 %
Metabolic Activation System plus Solvent Control	— : — X 100 = — %	— : — X 100 = — %
Cyclophosphamide Control (4.0 mg/ml)	40 : 40 X 100 = 100 %	— : — X 100 = — %
CO-Metabolic Activation System plus Test Material	0 : 40 X 100 = 0 %	14 : 40 X 100 = 35 %
MA + Solvent Control Length	mm	
Minimum Concentration to Inhibit Growth (MCIG)	MAS .15	NO MAS .20

TEST MATERIAL RESULTS

TEST	MORTALITY		MALFORMATION		STATISTICAL TEST USED
NOEL	MAS .25	NO MAS .35	MAS .12	NO MAS .15	BenFerroni T-test
LOEL	.28	.40	.15	.20	BenFerroni T-test
LC ₅₀	MAS .31	NO MAS .53	EC ₅₀	MAS .16	NO MAS .16
95% Confidence limits (MAS)		95% Confidence limits (NO MAS)	95% Confidence Limits		
(.29 - .32)		(.39 - .71)	MAS		NO MAS
			.15 - .17		.15 - .18
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)		MAS		NO MAS	
		1.94		3.31	

Offense NO MAS #2

FETAX with METABOLIC ACTIVATION (MA)
Summary Sheet

Test No. 2

Test Material	UNKNOWN P3M2	Investigator	TURLEY
Source	0.50	Lab	UMD-WREC
CAS No.		Lot No.	
Composition/Purity		Test Start Date	1/8/94
Solvent		Conc.	
		Test End Date	1/12/94
		Test Units (i.e., mg/ml)	mg/ml

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.35	7.42	7.37	7.38	—
Control	7.15	7.70	7.71	7.70	—
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed X 100 = %		
Total Number	0 : 40 X 100 = 0 %	1 : 40 X 100 = 2.5 %
Solvent Control	— : — X 100 = — %	— : — X 100 = — %
Metabolic Activation Control	0 : 40 X 100 = 0 %	2 : 40 X 100 = 5 %
Metabolic Activation System plus Solvent Control	— : — X 100 = — %	— : — X 100 = — %
Cyclophosphamide Control (4.0 mg/ml)	40 : 40 X 100 = 100 %	— : — X 100 = — %
CO-Metabolic Activation System plus Test Material	1 : 40 X 100 = 2.5 %	7 : 39 X 100 = 18 %
MA + Solvent Control Length	mm	
Minimum Concentration to Inhibit Growth (MCIG)	MAS .15	NO MAS .20

TEST MATERIAL RESULTS

TEST	MORTALITY		MALFORMATION		STATISTICAL TEST USED
NOEL	MAS .20	NO MAS .25	MAS .10	NO MAS .125	BenFurone, T-test
LOEL	.25	.30	.15	.15	BenFurone, T-test
LC ₅₀	MAS .25	NO MAS .53	EC ₅₀ .15	NO MAS .17	
95% Confidence limits	(.24-.26)	(.49-.57)	95% Confidence Limits	.13-.16	NO MAS .13-.22
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)					MAS 1.67 NO MAS 3.12

Coffeine w MAS #3

FETAX with METABOLIC ACTIVATION (MA)
Summary Sheet

Test No. 3

Test Material	UNKNOWN P3M2	Investigator	TURLEY
Source	SSU	Lab	UMD-WREC
CAS No.		Test Start Date	1/5/94
Composition/Purity		Test End Date	1/9/94
Solvent		Conc.	
		Test Units (i.e., mg/ml)	ml

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.39	7.43	7.45	7.40	—
Control	7.70	7.65	7.70	7.69	—
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %		
Solvent Control	0 : 40 X 100 = 0 %	3 : 40 X 100 = 7.5 %
Metabolic Activation Control	0 : 40 X 100 = 0 %	4 : 40 X 100 = 10.0 %
Metabolic Activation System plus Solvent Control		
Cyclophosphamide Control (4.0 mg/ml)	40 : 40 X 100 = 100 %	— : — X 100 = — %
CO-Metabolic Activation System plus Test Material	1 : 40 X 100 = 2.5 %	12 : 39 X 100 = 30.8 %
MA + Solvent Control Length	mm	
Minimum Concentration to Inhibit Growth (MCIG)	MAS .12	NO MAS .15

TEST MATERIAL RESULTS

TEST MATERIAL RESULTS					
TEST	MORTALITY		MALFORMATION		STATISTICAL TEST USED
NOEL	MAS .15	NO MAS .35	MAS —	NO MAS —	Bonferroni T-test
LOEL	.10	.40	.10	.10	Bonferroni T-test
LC ₅₀	MAS 0.20	NO MAS 0.49	EC ₅₀	MAS .12	NO MAS .13
95% Confidence limits	MAS (.20 - .21)	NO MAS (.46 - .53)	95% Confidence Limits	MAS .11 - .14	NO MAS (.11 - .14)
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			MAS 1.67	NO MAS 3.77	

FETAX with METABOLIC ACTIVATION (MA) *no MAS #1*
Summary Sheet *Colpene no MAS*

Test Material: P3M2		Test No. 1	
Source:		Investigator: DAWSON	
CAS No.		Lab: ASHLAND	
Lot No.		Start Date: 6-29-94	
Composition /Purity:		End Date: 7-3-94	
Solvent		Units: mg/ml	
-pH-	Day 0	Day 1	Day 3
Stock			Day 4
Control	X		Day 5
Highest Conc.	X		X

CONTROLS

Control	Mortality Record	Malformation Record
FETAX	$\frac{0}{80} \times 100 = 0\%$	$\frac{5}{80} \times 100 = 6.3\%$
Solvent	$\frac{\quad}{\quad} \times 100 = \quad\%$	$\frac{\quad}{\quad} \times 100 = \quad\%$
Metabolic Activation (MA)	$\frac{0}{40} \times 100 = 0\%$	$\frac{3}{40} \times 100 = 7.5\%$
MA + Solvent	$\frac{\quad}{\quad} \times 100 = \quad\%$	$\frac{\quad}{\quad} \times 100 = \quad\%$
Cyclophosphamide (+)	$\frac{40}{40} \times 100 = 100\%$	$\frac{\quad}{\quad} \times 100 = \quad\%$
Cyclophosphamide (-)	$\frac{0}{40} \times 100 = 0\%$	$\frac{2}{40} \times 100 = 5\%$
CO-MA + test material	$\frac{\quad}{\quad} \times 100 = \quad\%$	$\frac{\quad}{\quad} \times 100 = \quad\%$

LENGTH DATA

MA + Solvent Control length	mm
Minimum Concentration to Inhibit Growth (MCIG)	

TEST MATERIAL RESULTS

Test	Mortality		Malformation		Statistical Test Used
	no MAS	MAS	no MAS	MAS	
N ₀ EOEL	0.15	0.18	0.1	0.1	T-test
L ₀ EOEL	0.2	0.2	0.125	0.12	T-test

LC50 (95% confidence limits)		EC50 (95% confidence limits)	
no MAS	MAS	no MAS	MAS
0.402	0.262	0.144	0.143
0.362 - 0.446	0.248 - 0.278	0.131 - 0.159	0.132 - 0.155
TI - no MAS (95% confidence limits)		TI - MAS (95% confidence limits)	
2.78		1.83	
2.41 - 3.21		1.66 - 2.02	

FETAX with METABOLIC ACTIVATION (MA) *w/o MAS*
Summary Sheet *Caffeine w MAS #2*

Test Material: P3M2		Test No. 2	
Source:		Investigator: Dawson	
CAS No.	Lot No.	Lab: Ashland	
Composition /Purity:		Start Date: 7-5-94	
Solvent		End Date: 7-9-94	
Conc.:		Units: mg/ml	

-pH-	Day 0	Day 1	Day 3	Day 4	Day 5
Stock					X
Control	X				
Highest Conc.	X				

CONTROLS

Control	Mortality Record	Malformation Record
FETAX	$\frac{0}{80} \times 100 = 0\%$	$\frac{2}{80} \times 100 = 2.5\%$
Solvent	$\frac{\quad}{\quad} \times 100 = \quad\%$	$\frac{\quad}{\quad} \times 100 = \quad\%$
Metabolic Activation (MA)	$\frac{0}{40} \times 100 = 0\%$	$\frac{1}{40} \times 100 = 2.5\%$
MA + Solvent	$\frac{\quad}{\quad} \times 100 = \quad\%$	$\frac{\quad}{\quad} \times 100 = \quad\%$
Cyclophosphamide (+)	$\frac{40}{40} \times 100 = 100\%$	$\frac{\quad}{\quad} \times 100 = \quad\%$
Cyclophosphamide (-)	$\frac{0}{40} \times 100 = 0\%$	$\frac{1}{40} \times 100 = 2.5\%$
CO-MA + test material	$\frac{\quad}{\quad} \times 100 = \quad\%$	$\frac{\quad}{\quad} \times 100 = \quad\%$

LENGTH DATA

MA + Solvent Control length	mm
Minimum Concentration to Inhibit Growth (MCIG)	

TEST MATERIAL RESULTS

Test	Mortality		Malformation		Statistical Test Used
	no MAS	MAS	no MAS	MAS	
NEOEL	0.25	0.18	0.1	0.1	
LOEL	0.3	0.2	0.125	0.125	

LC50 (95% confidence limits)		EC50 (95% confidence limits)	
no MAS	MAS	no MAS	MAS
0.447	0.244	0.157	0.138
0.409 - 0.489	0.23 - 0.259	0.144 - 0.171	0.127 - 0.149
TI - no MAS (95% confidence limits)		TI - MAS (95% confidence limits)	
2.85		1.77	
2.52 - 3.23		1.61 - 1.95	

FETAX Summary Sheet

Cofferd w/ MAS #3

Test Material <i>P3M2</i>		Investigator	Doug Dawson
Source		Laboratory	Ashland
CAS No.	Lot No.	Test Start Date:	7/12/94
Composition/Purity		Test End Date	7/16/94
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7.3	7.27	7.26	7.3	
Control		7.44	7.35	7.3	7.27
Highest Concentration		7.51 / 7.	7.35 / 7.2	7.31 / 7.2	K13

No. Dead or Malformed		
X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	0 : 80 X 100 = 0%	3 : 80 X 100 = 3.8%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm) C20	Solvent Control Length (mm) J20	
Minimum Concentration to Inhibit Growth (MCIG) G21 0.1 0.1 w/MAS mg/ml		

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.35	0.1	T-test
LOEL	0.4	0.125	T-test
LC50	0.474	EC50 0.162	
95% CL	0.431 -- 0.522	95% Confidence limits 0.148 ---- 0.178	
Test Teratogenic Index (TI = LC50/EC50):			2.92
95% Confidence limits			2.55 -- 3.34

Percent effect	LC	EC
5	0.3302842	0.109
16	0.3810961	0.128
50	0.474308	0.162
84	0.5903186	0.207
95	0.681135	0.242

Coffene w MAS #3

FETAX Summary Sheet

Test No. 3

Test Material	P3M2-MAS	Investigator	Doug Dawson
Source		Laboratory	Ashland
CAS No.	Lot No.	Test Start Date:	7/12/94
Composition/Purity		Test End Date	7/16/94
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.3	7.27	7.26	7.3	
Control		7.44	7.35	7.3	7.27
Highest Concentration		7.51 / 7.	7.35 / 7.2	7.31 / 7.2	K13

No. Dead or Malformed		
X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	0 : 80 X 100 = 0%	3 : 80 X 100 = 3.8%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm) C20	Solvent Control Length (mm) J20	
Minimum Concentration to Inhibit Growth (MCIG) G21	0.1	mg/ml

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.18	0.1	T-test
LOEL	0.2	0.12	T-test
LC50	0.223	EC50	0.160
95% CL	0.213 -- 0.233	95% Confidence limits	0.149 ---- 0.172
Test Teratogenic Index (TI = LC50/EC50):		1.39	
95% Confidence limits		1.28 --	1.52

Percent effect	LC	EC
5	0.1851617	0.118
16	0.1992088	0.133
50	0.2227756	0.16
84	0.2491304	0.192
95	0.2680305	0.216

This test set up with Lot 8 microsomes
 Buffers Coffee w/ MMS #1

FETAX SUMMARY SHEET

Test Material P3M2		Test No. 4
Source		Investigator M. JACKSON
CAS No.	Lot No.	Lab STOVER
Composition/Purity		Test Start Date 2/15/94
Solvent		Test End Date 2/19/94
Conc.		Test Units (i.e., mg/ml)

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
pH					
Stock	7.4	7.4	7.5	7.5	
Control	8.0	8.0	8.0	8.0	
Highest Conc.	7.5	7.5	7.5	7.5	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed X 100 = %		
Total Number	$0 : 80 \times 100 = 0\%$	$0 : 80 \times 100 = 0\%$
Solvent Control	_____ X 100 = _____ %	_____ X 100 = _____ %
Control Length 89.8 ^{FAP} _{mm}	Solvent Control Length 84.7 ^{MMS} _{mm}	
Minimum Concentration to Inhibit Growth (MCIG) 0.1 uact		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.4	0.125	CONTROL Tox STAT
LOEL	0.5	0.15	Tox STAT
LC ₅₀	0.57 ^{uact}	0.29 ^{act}	EC ₅₀ 0.18 ^{uact} 0.10 ^{act}
95% Confidence Limits	0.55 - 0.60	0.25 - 0.29	95% Confidence Limits 0.19 - 0.19 0.10 - 0.11
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀) 3.17 ^{uact} 2.64 ^{act}			

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 = _____ %

FORK LIVING W W O M A S

#2

FETAX SUMMARY SHEET

Test Material <u>P3H2</u>		Test No. <u>1</u>
Source		Investigator <u>M. JACKSON</u>
CAS No.	Lot No.	Lab <u>FORT / STOVER</u>
Composition/Purity		Test Start Date <u>1/18/94</u>
Solvent	Conc.	Test End Date <u>1/22/94</u>
		Test Units (i.e., mg/ml)

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
<u>pH</u>					
Stock	7.5	7.7	7.5	7.6	
Control	8.0	7.9	7.9	8.0	
Highest Conc.	7.8	7.9	7.9	7.9	

FETAX CONTROL		MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed X 100 = %			
Total Number		0 : 80 X 100 = 0 %	12 : 80 X 100 = 15.0 %
Solvent Control		_____ : _____ X 100 = _____ %	_____ : _____ X 100 = _____ %
Control Length 83.1 mm		Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG)		0.1 / 0.1	

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>—</u>	<u>—</u>	<u>Failed Assumptions</u>
LOEL	<u>—</u>	<u>—</u>	
LC ₅₀	<u>0.671</u> / <u>0.260</u>	EC ₅₀	<u>0.133</u> / <u>0.1</u>
95% Confidence limits <u>0.66 - 0.69</u> / <u>0.26 - 0.27</u>		95% Confidence Limits <u>0.12 - 0.14</u>	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			<u>5.05</u> / <u>2.6</u>

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

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

Caffeine w MAS #3

FETAX SUMMARY SHEET

Test Material <u>P3M2</u>		Investigator <u>H. JACKSON</u>
Source		Lab <u>STOVER</u>
CAS No.	Lot No.	Test Start Date <u>2/8/94</u>
Composition/Purity		Test End Date <u>2/12/94</u>
Solvent	Conc.	Test Units (i.e., mg/ml)

— pH —	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock	7.5	7.5	7.6	7.7	
Control	8.0	8.0	8.1	8.1	
Highest Conc.	7.6	7.6	7.7	7.7	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %	<u>0</u> : <u>40</u> X 100 = <u>0</u> %	<u>0</u> : <u>40</u> X 100 = <u>0</u> %
Solvent Control	____ : ____ X 100 = ____ %	____ : ____ X 100 = ____ %
Control Length <u>88.7</u> mm <u>MAS</u> <u>77.2</u>	Solvent Control Length _____ mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>0.1 uACT</u> <u>uACT</u> (activated)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>0.35</u> <u>0.35</u> CONTROL	<u>0.12</u>	<u>TOXSTAT</u>
LOEL	<u>0.4</u> <u>0.4</u>	<u>0.1</u> <u>0.15</u>	<u>TOXSTAT</u>
LC ₅₀	<u>0.63 uACT</u> <u>0.37 uCT</u>	EC ₅₀ <u>0.18 uACT</u> <u>0.15 uCT</u>	
95% Confidence limits <u>.60 - .66</u> <u>.36 - .37</u>		95% Confidence Limits <u>.17 - .19</u> <u>.14 - .16</u>	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 3.33 uACT uCT

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 = ____ %

**PHASE III INTERLABORATORY STUDY OF FETAX;
PART 3- FETAX VALIDATION USING TWELVE
COMPOUNDS WITH AND WITHOUT AN EXOGENOUS
METABOLIC ACTIVATION SYSTEM**

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RUNNING HEAD: PHASE III-Part 3 ILS of FETAX

KEYWORDS: FETAX, *Xenopus*, teratogen, interlaboratory validation,
developmental toxicity

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ABSTRACT

FETAX (Frog Embryo Teratogenesis Assay-*Xenopus*) is a 96-h whole embryo developmental toxicity screening assay that can be used in ecotoxicology and in detecting mammalian developmental toxicants when an *in vitro* metabolic activation system is employed. A standardized American Society for Testing and Materials (ASTM) guide for the conduct of FETAX has been published along with a companion atlas that helps in embryo staging and in identifying malformations. As part of the ASTM process, an interlaboratory validation study was undertaken to evaluate the repeatability and reliability of FETAX and to evaluate the potential teratogenic hazard of twelve compounds. Three different laboratories participated in the study. All three participating laboratories had extensive experience with the assay. FETAX intralaboratory and interlaboratory variability, as judged by coefficients of variation, were very low. Potential teratogenic hazard was evaluated using two major criteria from FETAX experiments employing metabolic activation systems (MAS). These were the TI (teratogenic index = 96-h LC50 / 96-h EC50 (malformation)) and the Minimum Concentration that Inhibits Growth (MCIG). A compound was considered teratogenic by this criterion when the MCIG was significantly different from controls at concentrations below the 30% level of the MAS 96-h LC50. Based on the results of this and other studies, a decision table was constructed in order to evaluate additional studies. Severity of malformations caused, especially near the MAS 96-h EC50 (malformation) were also evaluated. Four compounds were non-teratogenic, while two compounds were clearly teratogenic. The remaining six compounds were ranked as equivocal teratogens. The results were discussed in light of the difficulty of producing an adequate decision table. FETAX proved to yield repeatable and reliable data as long as care was taken during range finding and technicians were adequately trained. The metabolic activation system was essential in using FETAX to predict developmental hazard in mammals and still requires further development.

INTRODUCTION

FETAX (Frog Embryo Teratogenesis Assay- *Xenopus*) is a 96-h whole embryo developmental toxicity test that utilizes the embryos of the South African clawed frog, *Xenopus laevis*. FETAX was initially designed as an indicator of potential human developmental health hazards and this use has been enhanced by the development of an *in vitro* metabolic activation system (MAS) using Aroclor 1254- and isoniazid-induced rat liver microsomes.¹⁻¹² FETAX has undergone extensive validation using single chemicals of known mammalian developmental toxicity and research continues on this alternative developmental toxicity test.

FETAX is also applicable to aquatic toxicity assessments and is well suited for testing complex mixtures such as industrial effluents or mixtures found at hazardous waste sites.¹³⁻¹⁶ Recent modifications have been made to FETAX to allow routine testing of volatile organics, soils and sediments¹⁷

FETAX may help in studies designed to discover the reasons for the reported world-wide disappearance of amphibians even in pristine locations.^{18,19} This decline may be due in part to normal population fluctuations caused by climatologic factors or by anthropogenic factors.²⁰ However, in at least one case, frog eggs failed to develop in pond water but developed normally when moved to the laboratory.²¹ It is, therefore, possible that some decline may be due to chemical pollution and FETAX may be used to investigate the extent and causes of the decline.

An American Society for Testing and Materials *New Standard Guide for the Conduct of FETAX* was published along with a companion *Atlas of Abnormalities* that aids in embryo staging and identifying malformations.²²⁻²³ Additional descriptions of FETAX have also been published that describe its use as a developmental toxicity screening test.^{1,3,10}

As part of the ASTM process, an interlaboratory validation study (ILS) was undertaken to determine the repeatability and reliability of FETAX. Secondary goals were the improvement of the FETAX protocol and the testing of additional compounds whose mammalian developmental toxicity was known. A three-phase experimental plan with seven participants was designed. Phase I was a training and protocol evaluation phase in which the identity of the three test materials was known. Because they had previously been tested in FETAX, the same concentrations needed to establish the 96-h LC50 and EC50 (malformation) were used by all laboratories. Phase I²⁴ showed that proper technician training was important in obtaining repeatable and reliable data. Several protocol changes were necessitated because of Phase I. Phase I results in terms of variability and correspondence to historical data were very good with only occasional high variation observed in some laboratories. Phase II was designed to be similar to Phase I except that the identity of the test materials was not known²⁵. All technicians had greater experience in Phase II than in Phase I and the nature of the test compounds may have played a role in the excellent results obtained in Phase II. Phase II showed far less intra- and interlaboratory variability than Phase I. Nonteratogens showed the most consistent results while more variability was observed for the two

teratogens tested. Interlaboratory coefficient of variation values for all FETAX endpoints ranged from 7.3 to 54.7%. The most variable endpoint was the MCIG and the least variable was the LC50. Phase III-Part 1 was designed to test FETAX using six test compounds in a blind testing format.¹¹ Each laboratory determined the concentrations to be tested. Results indicated that although generally acceptable data were obtained, a new protocol for range finding was needed so that repeatable and reliable results could be obtained. The *in vitro* metabolic activation system for FETAX was not employed in either Phases I, II or III-Part 1. In Phase III-Part 2, two compounds were tested using a metabolic activation system employing Aroclor 1254-induced rat liver microsomes.¹² The experimental design approximated Phase II when the compounds had previously been tested in FETAX and the test concentrations provided to each laboratory. The samples were coded and contained one compound activated by the microsomes and one that was deactivated. Results indicated that excellent, although slightly more variable results, could be obtained when the embryos were co-cultured with the MAS.

In this study, three laboratories, all very experienced in performing FETAX, tested 12 coded chemicals with and without Aroclor 1254-induced microsomes and each laboratory was responsible for determining tests concentrations. Following data analysis, a decision table was prepared for assessing the possible mammalian developmental toxicity hazard of each chemical. These results were then compared to tests performed using mammals.

MATERIALS AND METHODS

Interlaboratory Study Design and Protocol

Each laboratory participating in this interlaboratory study included a principal investigator and a primary technician. Three different laboratories participated in the study of twelve chemicals. Each laboratory technician performed FETAX testing while the principal investigators compiled, interpreted and reported the data to a central coordinator. The experimental results were screened by the coordinator to ensure they complied with the standard protocol established in the ASTM Standard Guide.²²

Rat Liver Microsome Preparation

Aroclor 1254-induced rat liver microsomes were prepared as described previously.⁵ Five days prior to microsome preparation, male Sprague-Dawley rats were injected with 500 mg/Kg Aroclor 1254 i.p. in corn oil.²⁶ Microsomal P-450 activity was estimated by measuring the N-demethylation of aminopyrine to formaldehyde using the methods of Lucier et al.²⁷ and Nash²⁸ as described previously.⁵ Protein was determined by the method of Bradford²⁹ (BioRad®, Richmond, CA). All rat liver microsomes were prepared by Dr. Bantle's laboratory and shipped frozen on dry ice to each of the participating laboratories. Prior to shipment, each lot of microsomes was diluted with Tris-HCl buffer (pH 7.5) to an appropriate N-demethylase activity so that each laboratory would effectively use the same activity throughout the study. To verify further the activity, each lot of microsomes was tested by co-culturing with embryos and 4.0

mg/mL cyclophosphamide which should induce 100% embryo lethality.

Following arrival, each laboratory stored the microsomal preparations in liquid nitrogen until needed.⁵ The metabolic activation system (MAS) included the microsomes and a NADPH generating system.

Animal Care and Breeding

Xenopus culture, breeding procedures and egg sorting were described previously in the ASTM Standard Guide²² and the Atlas of Abnormalities.²³ Adult *Xenopus laevis* frogs were obtained from Xenopus I (Ann Arbor, MI) or Xenopus Express (Beverly Hills, FL).

Test Compounds and Assay Protocol

Chemicals tested in this portion of the Phase III interlaboratory validation study were sodium arsenite (CAS# 7784-46-5), boric acid (CAS# 10043-35-3), ethylene glycol (CAS# 107-21-1), glycerol (CAS# 56-81-5), sodium iodoacetate (CAS# 305-53-3), acrylamide (CAS# 79-06-1), triethylene glycol dimethylether (CAS# 112-49-2), diethylene glycol (CAS# 111-46-6), phthalic acid (CAS# 877-24-7), dichloroacetate (CAS# 79-43-6), sodium bromate (CAS# 7789-38-0) and tribromoacetic acid (CAS# 75-96-7). The chemicals were purchased from Sigma Chemical Company® (St. Louis, MO) and Aldrich® (Milwaukee, WI) in bulk quantities from the same lot. Chemicals were coded, packed in labeled serum vials with hermetically sealed cups, sent to each laboratory and included a Material Safety Data Sheet sealed in an envelope which was available for use in an emergency.

Tests were performed as specified in the ASTM Standard Guide.²²

Technicians conducted several experiments to determine the chemical's effective range. From this collected data, EC5, EC16, EC50, EC84 and EC95 values were calculated for both the malformation and mortality curves. These specific calculated values were then used in the subsequent tests to expose *Xenopus* larvae to each individual chemical both with and without microsomes, simultaneously.

At the end of the experiment, the larvae were preserved in either 3% w/v paraformaldehyde or 3% v/v formalin, stored in glass vials (RPI®, Elkhart, IL) and were available to be sent to Dr. Bantle's laboratory for verification of results, if necessary.

Groups of 20 embryos were placed in 60-mm covered, plastic Petri dishes (Fisher Scientific, Houston, TX) with varying constant concentrations of chemical. Each chemical was reconstituted with FETAX-AB Solution (AB=antibiotics), a reconstituted water medium suitable for the culture of *Xenopus* embryos that included 100 U/mL penicillin- 100 U/mL streptomycin, to help control bacterial contamination. Ten test concentrations, tested in duplicate, were performed with and without the MAS. Each MAS treatment received 0.4 U/dish of aminopyrine N-demethylase activity and a NADPH generating system. Each treatment was prepared in a 50 mL Erlenmeyer flask and each dish received a total volume of 8 mL of solution.

Controls included four dishes with FETAX-AB solution (negative controls), four with FETAX solution alone, two dishes with MAS, two dishes

with activated cyclophosphamide (FETAX reference proteratogen), two dishes of the LC50 concentration with microsomes alone and two dishes of the LC50 concentration with the NADPH generating system alone. The latter two controls detected possible interactions between MAS components and test compound. These controls were tested simultaneously with each experiment.

Three separate definitive concentration-response tests with and without MAS were performed by each laboratory for all test compounds. Three experiments were performed in this manner with separate clutches of embryos and/or different lots of microsomes.

Criteria for Judging Development Hazard

The criteria for judging development hazard generally followed those previously proposed by Bantle.¹⁰ They include the consideration of the TI [Teratogenic Index; $TI = 96\text{-h LC50} / 96\text{-h EC50 (malformation)}$] and whether growth was significantly inhibited at concentrations less than 30% of the MAS 96-h LC50. Since comparison to mammalian data was desired in this study, data collected from tests performed with MAS were used in the comparisons since this exposure regimen most closely simulated mammalian development. When FETAX is used to assess mammalian developmental hazard, those tests employing the rat MAS most nearly approximate mammalian development. Proteratogens would be bioactivated and other compounds deactivated just as they are in mammals. Thus, the FETAX-MAS experiments formed the basis of assessing the developmental hazard.

Because a total of nine separate tests were performed by different laboratories, all the data were compiled and the interlaboratory mean TI and MCIG values were used in judging developmental hazards. Based on the criteria, each chemical was judged to have developmental hazard when both criteria indicated hazard and definitely not hazardous when both criteria fell into the non-hazard category. The hazard was considered equivocal when any one of the two criteria suggested hazard. In these cases, types and severity of malformations caused were examined for guidance in assessing teratogenic hazard. However, due to the subjectivity of the estimation, it was not made a permanent part of the decision criteria. Percent coefficients of variation were used to estimate the repeatability and reliability of the tests but the data collected from only three laboratories was not directly comparable to previous studies where a full six laboratories provided data.^{11, 12, 24, 25}

Because nine tests were performed, the MAS TI was considered to indicate hazard when the mean interlaboratory mean exceeded 1.5. The MAS MCIG was used to assess hazard based on a function of the 96-h LC50. A decision table was then constructed based on these and other results for use in assessing teratogenic hazard in future studies. The severity of malformation was interpreted by Dr. John A. Bantle and Dr. Douglas J. Fort based on a survey of all data received and reviewing malformations obtained at different concentrations. Malformations had to be increasingly severe and consistent as concentrations increased.

Data Analysis

Probit analysis, using the method of Litchfield-Wilcoxon, was used to determine the 96-h LC50 (median lethal concentration), 96-h EC50 (concentration inducing malformations in 50% of the surviving embryos), and 95% confidence intervals. When the homogeneity test failed, either the trimmed Spearman-Kärber, the EPA probit method³⁰ or a graphical method was used instead of the Litchfield-Wilcoxon³¹ probit analysis. Teratogenic hazard was determined using a teratogenic index [TI=96-h LC50/EC50 (malformation)]. Head-tail length (growth) was measured using an IBM-compatible computer equipped with digitizing software (Jandel Scientific, Corte Madera, CA). For each test, the MCIG was calculated using the t-test for grouped observations ($P < 0.05$). The coefficient of variation (CV) values for the 96-h LC50, 96-h EC50, TI, and MCIG were calculated according to Steel and Torrie.³²

The ability of MAS to alter LC50 and EC50 (malformation) endpoint data was determined by comparing the 9 separate definite NO MAS experiments with the 9 definitive MAS experiments using the t-test for group observations ($P=0.05$).

RESULTS

Variability and Teratogenic Hazard Data

Intralaboratory CV values (a measure of repeatability) providing an indication of intralaboratory variability may be found in Tables 1-12. These tables also show intralaboratory mean endpoint data for the NO MAS and MAS 96-h LC50, 96-h EC50 (malformation), TI and MCIG. Table 13 shows the interlaboratory CV values (a measure of reliability) and mean intralaboratory endpoint data for the NO MAS and MAS mean 96-h LC50, 96-h EC50 (malformation), TI and MCIG. The end point data presented are the averages of the interlaboratory mean values. The interlaboratory mean TI values are found by taking the mean of each TI value from each Laboratory. The estimation of teratogenic hazard is shown in Table 14 and the consensus values are based only on the consideration of the MAS TI, severity of malformations with MAS and the MAS MCIG<30% of the LC50. Lastly, Table 15 shows which endpoint was most variable based on a consideration of the interlaboratory CV values (Table 13).

Sodium Arsenite

Variability

All intralaboratory CV values were below 60.6% (Table 1). The correspondence in data was close for all laboratories, but Laboratory 3 reported higher values for the No MAS 96-h LC50 and No MAS 96-h EC50 (malformation) values. This was not the case for MAS experiments.

Interlaboratory CV values were below 34.9% except for the MAS MCIG which was 73.1% (Table 13). The LC50 was the least variable endpoint for the No MAS

experiments but the EC50 (malformation) was less variable for the MAS experiments (Table 15).

Teratogenic Hazard

Table 13 shows the No MAS and MAS Interlaboratory values for all FETAX endpoints for sodium arsenite. Because the MAS TI of 1.35 was below 1.5, this compound was ranked as a non-teratogenic hazard on Table 14. When embryos were co-cultured with MAS, the reduction in the MAS 96-h LC50 and the MAS 96-h EC50 (malformation) from the respective No MAS endpoints was 2.26 for the LC50 and 2.8 for the EC50 (malformation). This change was significant at the $P=0.5$ level.

The MAS MCIG was not 30% or less of the MAS 96-h LC50 and, therefore, sodium arsenite did not pose a teratogenic hazard (Table 14). The consensus ranking showed that sodium arsenite posed no teratogenic hazard (Table 14).

Sodium arsenite at the MAS 96-h EC50 (malformation) caused only a few severe malformations mainly in the gut, head, face and eye regions. At higher concentrations near the 96-h LC50, the malformations were more common, but not severe. Sodium arsenite did not produce the type and severity of malformations typical of a teratogen.

Boric Acid

Variability

All intralaboratory CV values fell below 68.5% and the MCIG showed some of the largest intralaboratory variability values (Table 2). For the NO MAS and MAS

experiments, Laboratory 1 had consistently higher or lower values, for 96-h LC50 MAS and 96-h EC50 (malformation) values. The interlaboratory CV values ranged from 29.2-61.5%. (Table 13). As expected because of the experimental design, the MCIG values had the highest CVs (Table 13). Table 15 shows that the LC50 was least variable in NO MAS experiments, but the EC50 was the least variable in MAS experiments.

Teratogenic Hazard

Table 13 shows the interlaboratory mean values for FETAX endpoints with and without MAS. The MAS TI was 2.39 which resulted in boric acid being ranked as posing a teratogenic hazard in Table 14. When MAS was added to the culture, it resulted in a reduction of mean 96-h LC50 by 1.66 fold and 1.31 for the 96-h EC50 (malformation). The reduction was significant for the LC50 at the $P=0.05$ level, but not the EC50 (malformation).

The MAS MCIG was not 30% or less of the 96-h LC50 and boric acid did not pose a teratogenic hazard based on the MAS MCIG (Table 14). The consensus ranking for boric acid was equivocal because one of the two criteria was teratogenic while the other was not (Table 14).

Boric acid did produce some severe malformations at some concentrations tested, but at or near the MAS 96-h EC50 (malformation) most embryos were normal or showed only slight malformations. Other embryos at these concentrations showed moderate malformations. As the MAS 96-h LC50, all of the embryos were either moderately or severely malformed. Given that at least half of the embryos at the MAS 96-h EC50

(malformation) were not malformed or only slightly malformed and that all embryos were malformed at the LC50, it was judged that boric acid posed an equivocal teratogenic hazard.

Malformations at the MAS 96-h EC50 (malformation) included reduced eyes and some abnormal gut coiling. At higher concentrations, severe stunting was observed and at higher concentrations all organ systems were malformed. Except for ocular edema, there was little other edema or blistering observed. Some embryos showed an upwardly curved tail.

Ethylene Glycol

Variability

Table 3 shows that all of the CV values for ethylene glycol were below 39.5% except for one MAS MCIG for Laboratory 3 which was 78.5%. For NO MAS experiments, 96-h LC50 and 96-h EC50 (malformation) values were all very consistent (Table 3). Laboratory 2 had a MAS 96-h LC50 higher than laboratories 1 and 3 but the 96-h EC50 (malformation) was lower.

Interlaboratory CV values ranged from 19.1 to 47.7% for all endpoints (Table 13). Table 15 shows that interlaboratory variability for NO MAS and MAS experiments was lowest for the LC50 endpoint. The MCIG was highest for both NO MAS and MAS experiments (Table 15).

Teratogenic Hazard

The MAS TI for ethylene glycol was 2.3 which ranked ethylene glycol as teratogenic (Table 14). When MAS was added to the culture, there was almost no reduction in the 96-h LC50 and 96-h EC50 (malformation) endpoints (Table 13).

Since the MAS MCIG was not less than 30% of the MAS 96-h LC50, then ethylene glycol ranked as a non-teratogenic hazard. Ethylene glycol ranked as an equivocal teratogen as the TI indicated hazard while the MCIG did not. (Table 14).

Ethylene glycol indicated some teratogenic hazard based on the severity of malformations induced. At the MAS 96-h EC50 (malformation), there were a few embryos exhibiting slight malformations but most were moderately to severely malformed. At concentrations at the 96-h LC50, all surviving embryos were severely malformed and all organ systems were severely affected. Considerable stunting was observed at higher concentrations. Because of the severity of malformations at both the MAS 96-h EC50 (malformation) and the MAS 96-LC50, ethylene glycol was considered a teratogen.

Glycerol

Variability

The highest intralaboratory CV value for glycerol was only 49.8% for the MAS MCIG from Laboratory 3 (Table 4). For NO MAS and MAS experiments, Laboratory 2 generally recorded the highest endpoint values. Interlaboratory CV values ranged from 15.0% to 55.0% for the MAS MCIG (Table 13.). Inclusion of MAS had no significant effect on all endpoints. The EC50 (malformation) was the least variable for NO MAS and MAS experiments (Table 15).

Teratogenic Hazard

Interlaboratory mean 96-h LC50, 96-h EC50 (malformation), MCIG and TI values for NO MAS and MAS tests are found in Table 13. There was no difference between NO MAS and MAS experiments for the mean TI which was approximately 1.57 ranking glycerol as an equivocal teratogenic hazard (Table 14). MAS activation had no apparent effect on the 96-h LC50 or 96-h EC50 (malformation) values and no statistical difference was found (Table 13).

Because the MAS MCIG was not less than 30% of the MAS 96-h LC50, glycerol did not pose a teratogenic hazard based on this criterion (Table 14). Because the TI was at the 1.5 limit and the MCIG indicated no teratogenic hazard, the glycerol was ranked as a probable non-teratogen (Table 14).

At concentrations near the MAS 96-h EC50 (malformation), glycerol caused no or only slight malformations leading to a ranking as a non-teratogenic hazard. However, glycerol did cause severe malformations as concentrations approached the MAS 96-h LC50. Some stunting was observed. As concentrations increased, gut malformations including poor coiling increased in frequency. Face and eye malformations increased in a concentration-response pattern.

Sodium Iodoacetate

Variability

For sodium iodoacetate, Laboratory 1 reported a intralaboratory CV value of 116.8% for the NO MAS 96-h LC50 and 120.9% for the MAS 96-h EC50 (malformation) (Table 5). CV values for the other two Laboratories were more acceptable and ranged from 2.3 to 63.7%. Table 5 also shows that the MAS TI for Laboratory 3 was 2.41 which is much higher than the other two laboratories (Table 5). In some laboratories, the concentrations selected did not yield responses that allowed the calculation of the endpoint. The high variability seen in intralaboratory experiments was reflected in the interlaboratory results (Table 13). CV values over 75% were observed for the NO MAS TI, the MAS 96-h LC50, the MAS 96-h EC50 (malformation) and the MAS TI.

Teratogenic Hazard

Interlaboratory endpoint data is shown Table 13 and the NO MAS TI for sodium iodoacetate was 1.24 indicating no teratogenic hazard. MAS inclusion had no effect on the endpoints as judged by statistical analysis and the MAS TI was 0.99. This was despite a 2.1 fold reduction in the 96-h LC50 and a 1.02 fold increase in the 96-h EC50 (malformation). The high variability of results from Laboratory 1 may have been responsible for this result.

The MAS MCIG was not less than 30% of the MAS 96-h LC50 and, therefore, sodium iodoacetate was not ranked as a teratogenic hazard for this criterion in Table 14. Both criteria for sodium iodoacetate demonstrated no teratogenic hazard (Table 14).

Sodium iodoacetate was not considered to be a non-teratogen in terms of severity of malformation. Malformations observed were judged to be slight at the MAS 96-h EC50 (malformation) and the MAS 96-h LC50.

When malformations were observed, they were mainly a loosely coiled gut which could have been an indicator of developmental delay. Abnormal gut coiling increased in frequency and severity with increasing concentrations of sodium iodoacetate. There were also face and eye malformations observed at concentrations approaching the 96-h LC50.

Acrylamide

Variability

Intralaboratory variability and individual laboratory data is presented in Table 6. Intralaboratory CV values ranged from 0-65.2%. Laboratory 2 reported the highest MAS 96-h LC50, MAS 96-h EC50 (malformation), and MCIG values. TI values for MAS tests ranged from 3.55 to 5.51. Interlaboratory CV values were 21.4 to 75.6%, the highest values were reported for the NO MAS MCIG (Table 13). Table 15 shows that for acrylamide, the EC50 (malformation) was the least variable endpoint for NO MAS and MAS experiments.

Teratogenic Hazard

Table 13 shows the interlaboratory endpoint data for acrylamide. The TI for MAS experiments was 4.6, while for NO MAS tests it was 4.25 (Table 13). The high MAS TI ranked acrylamide as having strong teratogenic potential (Table 14). Addition of MAS to the experiment resulted in significant reductions of 1.27 fold for the 96-h LC50, and 1.39 fold for the 96-h EC50 (malformation).

For acrylamide, the MAS MCIG was less than 30% of the 96-h LC50. This qualified acrylamide as a teratogenic hazard by the MCIG criterion (Table 14). Because both criteria indicated a teratogenic hazard, the consensus ranking was that acrylamide presented a teratogenic hazard (Table 14).

Acrylamide induced severe malformations at concentrations just above the 96-h EC50 (malformation) but well below the 96-h LC50. At the 96-h EC50 (malformation), all embryos showed severe malformations and exceptional stunting. At concentrations at or just below the 96-h EC50 (malformation), affected embryos exhibited upwardly curved tails. The number of embryos exhibiting these malformations increased in a concentration-response fashion as concentrations increased.

In addition to upwardly curved tails, all organs were malformed with obvious ocular and pericardial edema present. Face and eye malformations were especially prevalent.

Triethylene Glycol Dimethylether

Variability

Extremely low intralaboratory CV values were reported many of the endpoints for triethylene glycol dimethylether (Table 7). The highest value reported was 48.4% for the NO MAS MCIG for Laboratory 2. All of the laboratories reported very similar endpoint values. Interlaboratory CV values were from 16.1 to only 48.3% (Table 13). The LC50 for NO MAS and MAS was the least variable endpoint for triethylene glycol dimethylether (Table 15).

Teratogenic Hazard

Triethylene glycol dimethylether had a NO MAS TI of 3.94 and a MAS TI of 2.97 (Table 13). Table 13 presents the rest of the interlaboratory endpoint data. When MAS is added, there is a significant reduction in the 96 h LC50 by 1.75 fold. However, there was a small but still significant reduction in the 96-h EC50 (malformation). Because the MAS TI is 2.97 triethylene glycol dimethylether is ranked as a teratogenic hazard (Table 14). Table 14 shows that triethylene glycol dimethylether was a teratogenic hazard by both criteria (Table 14), and, thus, its consensus ranking was that of a teratogen.

The MAS MCIG was less than 30% of the MAS 96 h LC50 indicating teratogenic hazard by this criterion (Table 13). Therefore, the consensus was that triethylene glycol dimethylether posed a teratogenic hazard. The LC50 was the least variable endpoint (Table 15).

Triethylene glycol dimethylether caused severe gut malformations at concentrations near the MAS 96-hr EC50 (malformation). At concentrations lower than this, malformations were often slight but some sensitive embryos were always malformed. As the MAS 96-hr EC50 (malformation) was approached, head, face and eye malformations were severe in all the embryos. The tail was generally recurved upward and the embryos were always stunted. Eye size was reduced in many embryos and some cyclopia was evident. Given the severity and consistency of malformations at the MAS 96-hr EC50 (malformation), this compound was a teratogen.

Diethylene Glycol

Variability

All intralaboratory CVs were at or below 68.1% and 22 of 24 CV values were at or below 46.8% indicating low variability in results (Table 8). For the 96-h EC50 (malformation) without MAS, the value for Laboratory 3 was half that of the other two laboratories as the upper confidence limit for Laboratory 3 was less than the lower limits for Laboratories 1 and 2. For the 96-h EC50 (malformation) with MAS, Laboratory 3 obtained a much lower value than the other two laboratories and the MAS MCIG was also much lower. The values were the only two that were markedly lower than the other two laboratories.

Interlaboratory CV values ranged from 16.4 to 79% (Table 13). Interlaboratory CV values were all at or below 65.9 except for the MAS MCIG (Table 13). The least variable endpoint for experiments with and without MAS was the LC50 (Table 15).

Teratogenic Hazard

Interlaboratory mean 96-h LC50, 96-h EC50 (malformation) MCIG and TI values for No MAS and MAS experiments are shown in Table 13. The interlaboratory MAS TI value equaled 2.5 which ranked this chemical as a teratogenic hazard in Table 14.

Inclusion of MAS, caused a mean reduction of the LC50 and EC50 values by only 1.25 fold. However, the addition of MAS caused a significant reduction of the 96-h LC50.

The MAS MCIG was 11.33 mg/mL and the MAS 96-h LC50 was 26.61 mg/mL (Table 13). Because the MCIG was greater than 30% of the 96-h LC50, diethylene glycol was ranked as a non-teratogen in Table 14. Based on both criteria, the consensus ranking was as an equivocal teratogenic hazard (Table 14).

At or near the MAS 96-h EC50 (malformation), diethylene glycol was capable of causing many moderate to severe malformations, However, nearly 30% remained slightly malformed. At concentrations at or near the MAS 96-h LC50, 90% of the malformations were ranked as severe. Because a number of malformations were slight to moderate at the MAS 96-h LC50, this compound was an equivocal teratogen although there was a tendency towards severe effects.

Malformations of the gut were always present and exceeded what could be expected if developmental delays were suspect. Face, head and eye malformations were common but edema and spinal kinking were rare.

Phthalic Acid

Variability

Intralaboratory CV values were generally quite low for phthalic acid, but the highest value was 84.3% for the MAS MCIG (Table 9). There was generally good correspondence in the mean intralaboratory endpoint values for the LC50, EC50 (malformation) and MCIG. Laboratory 3 reported a higher NO MAS TI of 2.51 which was higher than the other two laboratories, but this variation was not seen for the MAS

TI. Interlaboratory CV values ranged from 9.5-57.2% (Table 13). Table 15 shows that the least variable NO MAS endpoint was the LC50 while the most variable was EC50. For MAS experiments, the LC50 endpoint was the least variable. The MCIG endpoint was the most variable for MAS experiments.

Teratogenic Hazard

Endpoint values for phthalic acid are shown on Table 13. MAS addition reduced mortality and malformation endpoints by 1.35 with the LC50 reduction being insignificant. The mean interlaboratory MAS TI was 1.43 and indicated no hazard (Table 14).

The MAS MCIG was not 30% of the 96-hr LC50 and, therefore, was not ranked as a teratogenic hazard (Table 14). Thus, both measures indicated that phthalic acid posed no significant teratogenic hazard (Table 14).

At or near the MAS 96-h EC50 (malformation), phthalic acid caused only slight to moderate malformations. As concentrations approached the MAS 96-h LC50, more embryos were moderately to severely malformed although some embryos still showed only slight malformations. Although there were some embryos moderately malformed, at concentrations near the MAS 96-h EC50 (malformation), they were not severe enough to rank these compounds as teratogenic (Table 14). Malformations commonly occurred in the gut, face, eye and brain often accompanied by edema.

Dichloroacetate

Variability

Intralaboratory variation for dichloroacetate was similar to many of the other compounds in the study (Table 10). High values for Laboratory 2 were reported for the NO MAS and MAS MCIG values. All other CV values were at or below 32. Interlaboratory CV values were from 15.8 to 70.6%. Most of the CV values for the endpoints were above 40%. Laboratory 2 accounted for much of the variability as it recorded much lower values for the NO MAS and MAS 96-h EC50 (malformation). This resulted in much higher TI values than those recorded by the other two laboratories.

Teratogenic Hazard

Interlaboratory mean 96-h LC50, 96-h EC50 (malformation), MCIG and TI values for No MAS and MAS experiments are shown in Table 13. Addition of MAS significantly lowered LC50 values, but not EC50 (malformation) values (Table 13). The interlaboratory MAS TI value for dichloroacetate was 3.35 and indicated a teratogenic hazard (Table 14). The high variability as indicated by the CV values suggest caution in interpreting this result.

Table 13 shows that the MAS MCIG was more than 30% of the 96-h LC50 so it was not possible to rank dichloroacetate as teratogenic based on the growth criterion. The

TI indicated teratogenicity while the MCIG identified dichloroacetate as a non-teratogen (Table 14).

Dichloroacetate caused very few severe malformations at or near the MAS 96-h EC50 (malformation). Most of the embryos were quite normal in appearance. Some embryos were severely malformed while others were moderately malformed. At the MAS 96-h LC50, they were all severely malformed. At the lower concentrations, loose gut coiling was observed but at higher concentrations all organ systems were involved. Most of the observable malformations occurred in the face, eye and brain.

Sodium Bromate

Variability

Intralaboratory variation for sodium bromate was generally quite low. Only four values were above 50% (Table 11). Many of the other values were below 35%. All three laboratories reported very similar results for the NO MAS 96-h LC, 96-h EC50 (malformation), MCIG and the TI (Table 11). However, Laboratory 1 reported a low mean value of 0.18 mg/mL for the MAS 96-h LC50 while the other two laboratories were substantially higher. All three laboratories reported NO MAS TI values equal to or greater than 3.29, but treatment with MAS lowered this mean value substantially. The MAS TI values were 1.21, 3.2 and 2.44 for Laboratories 1, 2 and 3, respectively (Table 11). Interlaboratory variation for sodium bromate ranged from 25.5 to 84.8% (Table 13). Once again, the highest value for variation among the MAS endpoints was the MCIG

(75,4%). However, relatively high values were also observed for the MAS 96-h LC50 and the MAS 96-h EC50 (malformation) (Table 13). Based on interlaboratory CV values (Table 13), the LC50 was least variable for NO MAS experiments and the EC50 was least variable for MAS Experiments (Table 15).

Teratogenic Hazard

The interlaboratory values for the 96-h LC50, 96-h EC50 (malformation), MCIG and TI for NO MAS and MAS experiments are given in Table 13. The NO MAS TI value was 3.59, but the MAS TI value was 2.28 (Table 13). It was concluded based on the MAS TI that sodium bromate did pose a developmental hazard for malformation (Table 14).

The MAS MCIG was not equal to or less than 30% of the MAS 96-h LC50 so there was little hazard as judged by this criterion. Since one criterion was negative, and one indicating a strong teratogenic hazard, sodium bromate was judged to be equivocal (Table 14).

Sodium bromate caused some severe malformations at the MAS 96-h EC50 (malformation). There was considerable stunting in all embryos at or near the MAS and No MAS LC50 concentrations. At the LC50 for the No MAS and MAS-treated embryos, malformations were only moderate in nature. Because severe malformations were caused at the MAS 96-h EC50 malformation, it was concluded that sodium bromate did pose a teratogenic hazard as judged by the severity of malformations caused.

Tribromoacetic Acid

Variability

Intralaboratory variability for tribromoacetic acid was low for NO MAS experiments but moderate for MAS experiments (Table 12). Laboratory 3 showed lower values for 96-h LC50 and EC50 (malformation) endpoints for NO MAS and MAS experiments (Table 12). Interlaboratory variation ranged from 20.4 to 81.3% reflecting the higher intralaboratory variation reported from each of the three Laboratories (Table 13). Table 15 shows that the MCIG was least variable for NO MAS experiments and the LC50 was least variable for MAS experiments.

Teratogenic Hazard

Table 13 reports the interlaboratory endpoint data for tribromoacetic acid. MAS addition lowers the LC50 by 1.44 fold and the EC50 (malformation) by 1.2 fold. The reduction was not significant. The NO MAS TI was 3.86 but for MAS experiments the TI was 4.14 making tribromoacetic acid a teratogenic hazard by this criterion (Table 14).

The MAS MCIG was not equal to or less than 30% of the MAS 96-h LC50 and it was listed as non-teratogenic on Table 14. Given one of two criteria were teratogenic and the other was non-teratogenic, tribromoacetic acid was ranked as equivocal (Table 14).

Tribromoacetic acid caused severe malformation in some embryos. A number of

embryos were moderately malformed. However, there were embryos even at high concentrations that showed only slight malformations. The severe malformation rate was high enough to rank this as developmentally toxic. At concentrations near the MAS 96-h EC50 (malformation), face, eye and brain malformations were clearly the most evident. At the 96-h LC50, face, eye, brain and gut malformations were most prominent. Tribromoacetic acid was typical for a compound with a TI of 4-5 in that it caused severe malformations, but it was atypical in that some embryos were greatly affected while others were not.

DISCUSSION

FETAX Variability

Intralaboratory variation

This study differed from earlier ILS studies in that only three laboratories participated in the study. This made the comparison of interlaboratory variation difficult because six or seven laboratories took part in earlier studies. However, since each laboratory performed three definitive tests throughout all phases of the ILS, it was possible to compare intralaboratory results for the present study with the results from Phase II²⁵. The Phase II study had very low intralaboratory CV values compared to other phases of the FETAX ILS study. The mean Phase II intralaboratory CV values for the NO MAS 96-h LC50, 96-h EC50 (malformation) and NO MAS MCIG were 3.6, 12.3 and 20.8%, respectively²⁵. These values represented the means for all laboratories and all chemicals in the study. For Phase III, part 3, the mean intralaboratory CV values for the NO MAS 96-h LC50, 96-h EC50 (malformation) and NO MAS MCIG were 16.6, 17.1 and 22.9%, respectively. While very good compared to past FETAX results, Phase III, Part 3 showed slightly more intralaboratory variation than Phase II. The nature of the chemical tested and its developmental toxicity may play a role in the variation encountered in testing. There were also changes in technician staffing in some of the participating laboratories and this may have contributed to the variation observed. Lastly, all laboratories used the same concentrations ranges in Phase II, but determined their own concentration ranges in the present study. Inclusion of MAS led to an increase in intralaboratory variation of less than 1.5 fold. This was not a great increase considering the complexity of the metabolic activation system added.

Interlaboratory Variation

Because of the difference in the number of participating laboratories, direct comparison was difficult. Inspection of Table 13 suggested that variation is low, as evidenced by the low CV values. Most CV values were below the 75% that Parkhurst³³ suggested was excellent for studies involving six laboratories. The intralaboratory variation discussed above suggests that this study had more variation than Phase II²⁵ but less than Phase III, Part 1¹¹.

Table 15 shows that the LC50 was the least variable endpoint for NO MAS (eight of 12 chemicals tested) and MAS experiments (seven of 12 chemicals tested). The MCIG was the most variable endpoint. Because the concentration series was specifically designed to determine the 96-h LC50 and the 96-h EC50 (malformation), the MCIG would be expected to be more variable even though it is an objective endpoint. For all 27 chemicals tested in the entire FETAX interlaboratory study (Phase III, Parts 1-3 including MAS experiments), the LC50 was the least variable endpoint for 18 of 27 chemicals^{11, 12, 24, 25}. The EC50 (malformation) was least variable in nine cases and the MCIG was never the least variable endpoint. In contrast, The MCIG was the most variable endpoint in 19 of 27 chemicals tested. Clearly, the cessation of heartbeat as a clear indication of mortality allows for low variability in this endpoint. Adjusting test concentrations to better define the MCIG may lessen the variability of the MCIG in the future.

Predictive Ability of the Teratogenic Index

The TI has long been used as one predictor of teratogenic hazard. The normal procedure is to use the mean TI from three definitive experiments¹⁰. A value over 1.5 indicates teratogenic hazard and this relative hazard increases with increasing TI values. Semicarbizide has a TI value of 3000 indicating the higher end of this scale³⁴.

For this study, the mean MAS TI was used as one criterion to assess teratogenic hazard in an effort to increase the predictive accuracy of this measure and reduce false positives. However, FETAX is ordinarily performed by only a single laboratory. Therefore, when each chemical is evaluated solely on the basis of the mean MAS TI in each laboratory, boric acid, acrylamide, triethylene glycol dimethylether and diethylene glycol were found to be teratogenic hazards in all laboratories. Ethylene glycol, glycerol, dichloroacetate, sodium bromate and tribromoacetic acid were considered to be teratogenic hazards in two of three laboratories, and sodium arsenite, sodium iodoacetate, phthalic acid were determined to be teratogenic hazards in one of three laboratories. All chemicals were teratogenic in at least one laboratory. Thus, a degree of uncertainty was introduced for eight of 12 chemicals in the study. In other FETAX studies, results were less ambiguous even with six laboratories. However, there was some danger in just using the individual TI as the sole indicator of teratogenicity because of the variation for chemicals with a low TI. Therefore, we considered the MCIG in the decision criteria and severity of malformations caused before rendering a judgement.

Effect of Metabolic Activation

MAS addition to the FETAX test entailed adding microsomes, co-factors and enzymes. Even though amounts were adjusted so that embryos grew and developed normally, these components often stressed the embryos. Many past experiments show that some chemicals were bioactivated³⁵ to highly toxic compounds while others were deactivated by similar amounts⁴. When results from nine definitive tests from three separate laboratories were statistically analyzed, it was possible to determine when the results were significant as discussed in the results. NO MAS and MAS experiments were not directly comparable statistically because NO MAS experiments did not have microsomal protein and generator components added. However, when a single laboratory only performed three tests, there were usually some slight reductions in endpoint values that appear to be bioactivation. Although lacking in long-term experience with metabolic activation systems, it generally required at least a two-fold change in NO MAS endpoints to MAS endpoints before the bioactivation and deactivation was considered a real event given the potential variation in results. Given this "rule of thumb", only the 96-h LC50 was reduced two-fold for sodium bromate. Metabolic activation did not play a great role in this study in the consideration of developmental toxicity for the other chemicals.

Decision Criteria

FETAX data are often compared to mammalian developmental toxicity databases when attempting to make a link to human developmental toxicity. Problems abound with

this approach not the least of which is the unsuitability of the rodent models for human developmental toxicity studies. The yolk sac poisons in rodents are examples of false positives in the rodent model and thalidomide is an example of a false negative in the rodent system. Besides structural dissimilarities, differences in metabolism also lead to problems when interspecies comparisons are made. Between mammalian species, conflict also arise. For instance, ethylene glycol is teratogenic in rat³⁶ while it is not in rabbit³⁷. Ideally, FETAX data should be compared directly with human teratogenicity data where exposure information is available. Unfortunately, there is not enough reliable data to permit this for the over 100 compounds that should be tested as part of a validation study for predictive accuracy. The best current approach is to choose chemicals that have been tested using at least three concentrations in the rat, mouse and rabbit using standard techniques and the same exposure route. These chemicals should ideally have some human exposure data available. Only a few of the chemicals in this study meet these criteria. The mammalian consensus results shown in Table 14 represent the contribution of all relevant mouse, rat and rabbit oral exposure data that is currently available for these compounds. When a compound is listed "V" for variable in this part of Table 14, it is because of a positive teratogenic result in one species and a negative in another. To be listed as a positive, congenital malformations at concentrations lower than that required to cause maternal toxicity must have been recorded. Fetal weight loss at these concentrations does not qualify a compound to be listed as teratogenic. For the FETAX consensus, an "E" for equivocal is used when one value indicates teratogenic hazard, while the other does not.

An additional confounding factor is the route of exposure. In mammalian studies, the routes are typically oral, i.p. or i.m. injection, dermal and inhalation. For the commonly used oral exposure route in mammals, the test chemical may be broken down by digestion, not absorbed well by the gut or largely detoxified (or bioactivated) by the liver before even being presented to the embryo. In FETAX, exposure is dermal, but given the highly absorptive nature of amphibian skin, the actual exposure is both dermal and equivalent to intraperitoneal injection by nature. The route of exposure can greatly affect test results. Since most mammalian data available was oral exposure, this was chosen as the basis of comparison, although it was not best exposure route for this purpose.

In establishing the decision criteria table shown in Table 14, it was decided that more than one criterion should be utilized before ranking a compound a unequivocal mammalian teratogenic hazard. The problems discussed above regarding the variation of the intralaboratory mean TI value around the value of 1.5 suggested that other criteria should be used. The MCIG criteria presented some difficulty because the concentration ranges were really established to define the 96-h LC50 and 96-h EC50 (malformation) leading to an acceptable but more variable MCIG endpoint. Table 14 presents one example of a decision table where the two criteria must be met prior to ranking a compound as a teratogenic hazard. Prior experience with FETAX suggested this may be a viable way to interpret the data. One difference from standard FETAX procedure is that three participating laboratories were used, when, usually, only one laboratory would be performing experiments and interpreting data.

Comparison to Mammalian Data

Relationship of Phase III-Part 3 Compounds to Other Compounds Tested in FETAX

Table 16 shows a comparison of the TI values recorded for this study (both No MAS and MAS) with values obtained in Dr. Bantle's and Dr. Fort's laboratory using standard FETAX methodology. The comprehensive data set includes values from the present study. The TI for the present set of chemicals is concentrated between <1 and 5.0 (Table 16). For the comprehensive set of TI values, the TI values range from <1 to >600. Of the 109 TI values reported, some 30% were greater than 5. Compared to the range of FETAX compounds tested to date, the present set of 12 compounds represents a very narrow range of TI values and partially explains some of the ambiguity of results. With so many compounds having TI values near the 1.5 borderline, natural variation would cause many values to be above and below the 1.5 criterion. A set of compounds more representative of the suite already tested would have had far fewer borderline compounds.

FETAX Prediction of Teratogenic Risk Based on Decision Criteria

Of the 12 chemicals tested, eight posed a teratogenic hazard by ranking as either equivocal or teratogenic, four were non teratogenic in FETAX. Bantle¹⁰ proposed that a chemical posed a teratogenic hazard if any of the following criteria were met: 1.) the

mean TI was greater than 1.5, 2.) the malformations, especially near the 96-h EC50 (malformation) were severe, or 3.) the MCIG was less than 30% of the 96-h LC50. However, the severity of malformations caused was too subjective in practice, and this data was not used in making the assessment of teratogenic hazard. However, it was taken into consideration when the FETAX ranking was equivocal. If the chemicals are ranked according to Bantle¹⁰, then those posing a teratogenic hazard would be boric acid, ethylene glycol, acrylamide, triethylene glycol dimethyl ether, diethylene glycol, dichloroacetic acid, sodium bromate and tribromoacetic acid (Table 14). Most would be ranked because of the TI value exceeded 1.5. Glycerol was borderline in TI as the MAS TI was 1.66 (Table 13).

The results of mammalian developmental toxicity tests were obtained from the National Toxicology Program, the available scientific literature, two general papers on developmental toxicity screening tests and two reference source books³⁸⁻⁴¹. Only references to oral administration were used if a choice existed and only mouse, rat and rabbit data was employed. If any one species differed from the rest, the compounds was ranked as "V" or variable on Table 14.

Table 14. shows that when the FETAX consensus is compared to Mammalian consensus ratings, there are nine compounds that corresponded, two compounds that were false negatives (FN) in FETAX and one compound that was false positive (FP). This assumed that compounds that rated equivocal in FETAX corresponded to compounds that rated teratogenic or variable in mammals. When only the FETAX TI values were used to assess teratogenicity, nine compounds corresponded with excellent results. It may be necessary to adjust the test concentrations in order to obtain a more accurate MCIG that

will correlate better or it may be that the 30% of the MCIG criterion is too strict an estimator. Further research needs to be performed in order to assess which possibility is correct. It must be remembered that the TI values obtained for Phase III-P3 were relatively low and the compounds were not severe teratogens. The 30% of the MCIG standard worked very well for obvious teratogens and was derived from this work.

Sodium arsenite was a false negative in FETAX. In fact, sodium arsenite was more teratogenic and embryotoxic in mammals than sodium arsenate ⁴². Similarly, in FETAX the 96-h LC50 for sodium arsenite was 20 fold less than the 96-h LC50 for sodium arsenate and the 96-h EC50 (malformation) was 10 fold lower. Thus, sodium arsenite was quite embryotoxic and not teratogenic. Even the severity of malformations did not support a conclusion that sodium arsenite was teratogenic. Thus, the conclusion that sodium arsenite is a FETAX false negative cannot be changed due to an analysis of the severity of malformations.

Glycerol ranked as a FETAX false positive as the TI was just over the 1.5 cutoff. The conclusion was not supported by the MAS MCIG, but it was supported by inspection of the severity of malformations. Had the NO MAS TI been used (Table 13), glycerol would probably have been classified as a negative. Some adjustments to the MAS system may allow fewer false positives to be recorded.

Sodium iodoacetate was a false negative in FETAX but teratogenic in mammals ^{43,44}. For unknown reasons, this proved a difficult compound to test and several failures caused only partial data to be recorded (Table 5). Inspection of the severity of malformations caused revealed no basis upon which to base the conclusion that sodium iodoacetate was teratogenic. The NO MAS TI was also less than 1.5.

In conclusion, FETAX was 75% (9 of 12) accurate in judging whether a substance was teratogenic when compared to a combined mammalian data base. A previous summation of early validation studies with FETAX reached a conclusion that the correspondence was higher than 85%¹⁰. Clearly, Table 16 shows that most of the compounds chosen for this study were either weakly or variably teratogenic.

In formulating a superior decision table for future work, the mean MAS TI seems adequate in light of the nature of the test compounds. Experiments should be performed to determine whether the MCIG standard is set too high or whether the endpoint should be better defined by appropriate concentrations. The high variability for the MCIG as evidenced by Table 15 suggests more appropriate concentrations need to be used. Although the severity of malformation did not change any conclusions in the present study, it was useful in confirming conclusions and in defining which organ systems were affected.

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Phase III, Part 3

Tables

Chemical Codes

Code	Chemical	CAS #	Sigma Cat #
NA	Sodium Arsenite	7784-46-5	S 7400
NB	Boric Acid	10043-35-3	B 0252
NC	Ethylene Glycol	107-21-1	E 9129
ND	Glycerol	56-81-5	G 7893
NE	Sodium Iodoacetate	305-53-3	I 2512
NF	Acrylamide	79-06-1	A 8887
NG	Triethylene Glycol Dimethylether	112-49-2	T 3518
NH	Diethylene Glycol	111-46-6	D 3381
NI	Phthalic Acid	877-24-7	P 6758
NJ	Dichloroacetate	79-43-6	D 6399
NK	1,2 Dichloroethane	107-06-2	D 8026
NL	Sodium Bromate	7789-38-0	**S2487-1
NM	Tribromoacetic acid	75-96-7	**T4820-8

** purchased from Aldrich Chemical rather than Sigma

Table 1. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCIG and TI Values for Sodium Arsenite with Coefficients of Variation.

Laboratory	Without MAS*												With MAS											
	LC50			EC50			MCIG			TI			LC50			EC50			MCIG			TI		
	Mean (mg/mL)	CV (%)		Mean (mg/mL)	CV (%)		Mean (mg/mL)	CV (%)		Mean	CV		Mean (mg/mL)	CV (%)		Mean (mg/mL)	CV (%)		Mean (mg/mL)	CV (%)		Mean	CV	
	and 95% CI**									and 95% CI												and 95% CI		
1	0.106			0.100			0.060			1.05			0.061			0.038			0.040			1.62		
	0.087	14.8	0.089	0.094	15.3	0.088	0.060	0.067	14.1	0.93	1.02	6.4	0.042	0.055	16.4	0.050	0.043	11.9	0.040	0.040	0.0	0.84	1.31	25.7
	0.074	0.071 - 0.107		0.069	0.069 - 0.106		0.080	0.054 - 0.080		1.08	0.93 - 1.11		0.061	0.042 - 0.067		0.041	0.036 - 0.050		0.040	0.040 - 0.040		1.46	0.84 - 1.77	
2	0.090			0.072			0.065			1.25			0.052			0.033			0.034			1.58		
	0.102	0.105	13.4	0.077	9.0	0.079	0.065	0.069	8.8	1.32	1.32	4.3	0.053	0.053	0.9	0.039	0.035	8.9	NA	0.041	16.0	1.36	1.53	8.3
	0.124	0.086 - 0.125		0.089	0.069 - 0.089		0.078	0.061 - 0.078		1.39	1.24 - 1.40		0.053	0.052 - 0.053		0.032	0.030 - 0.039		0.047	0.030 - 0.051		1.66	1.36 - 1.71	
3	0.090			0.090			0.090			1.00			0.020			0.020			0.150			1.00		
	0.140	0.117	17.6	0.140	20.7	0.127	0.090	0.063	60.6	1.00	0.93	10.1	0.032	0.031	26.8	0.020	0.027	35.4	0.150	0.110	51.4	1.60	1.20	23.6

*MAS=Metabolic Activation System

**CI=Confidence Interval

Table 2. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCIG and TI Values for Boric Acid with Coefficients of Variation.

Laboratory	Without MAS*										With MAS									
	LC50					EC50					MCIG					TI				
	Mean (mg/mL) and 95% CI**	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)
1	1.365		0.548		0.400		0.400		0.400		0.400		0.400		0.400		0.400		0.400	
	1.010	12.9	0.462	7.1	0.400	0.0	0.400	0.0	0.400	0.0	0.400	0.0	0.400	0.0	0.400	0.0	0.400	0.0	0.400	0.0
	1.110	0.955 - 1.369	0.525	0.461 - 0.562	0.400	0.512	0.461 - 0.562	0.400	0.512	0.461 - 0.562	0.400	0.512	0.461 - 0.562	0.400	0.512	0.461 - 0.562	0.400	0.512	0.461 - 0.562	0.400
2	1.217		0.226		0.100		0.100		0.100		0.100		0.100		0.100		0.100		0.100	
	1.075	6.7	0.166	14.0	0.100	0.0	0.100	0.0	0.100	0.0	0.100	0.0	0.100	0.0	0.100	0.0	0.100	0.0	0.100	0.0
	1.046	1.009 - 1.216	0.175	0.152 - 0.226	0.100	0.189	0.152 - 0.226	0.100	0.189	0.152 - 0.226	0.100	0.189	0.152 - 0.226	0.100	0.189	0.152 - 0.226	0.100	0.189	0.152 - 0.226	0.100
3	1.010		0.520		0.015		0.015		0.015		0.015		0.015		0.015		0.015		0.015	
	0.420	38.4	0.270	36.6	0.250	64.5	0.250	64.5	0.250	64.5	0.250	64.5	0.250	64.5	0.250	64.5	0.250	64.5	0.250	64.5
	0.550	0.309 - 1.011	0.240	0.169 - 0.517	0.250	0.343	0.169 - 0.517	0.250	0.343	0.169 - 0.517	0.250	0.343	0.169 - 0.517	0.250	0.343	0.169 - 0.517	0.250	0.343	0.169 - 0.517	0.250

*MAS=Metabolic Activation System

**CI=Confidence Interval

Table 3. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCIG and TI Values for Ethylene Glycol with Coefficients of Variation.

Laboratory	Without MAS*						With MAS					
	LC50			EC50			MCIG			TI		
	Mean (mg/mL)	CV (%)	95% CI**	Mean (mg/mL)	CV (%)	95% CI	Mean (mg/mL)	CV (%)	95% CI	Mean	CV (%)	95% CI
1	22.04	12.23	8.33	12.23	3.7	12.50	10.64	16.3	1.45	1.61	8.9	19.70
	19.21	20.26	6.3	13.22	12.37	1.45	10.64	16.3	1.45	1.61	8.9	16.85
	19.53	18.504	22.019	12.25	11.929	13.205	11.10	8.242	13.043	1.59	1.414	22.47
												11.59
2	34.06	10.24	15.00	10.24	2.2	15.00	15.00	0.0	2.54	2.97	11.0	11.54
	27.35	31.00	8.9	10.78	10.47	10.784	15.00	0.0	2.54	2.97	11.0	11.57
	31.58	27.157	34.836	10.38	10.150	10.784	15.00	0.0	2.54	2.97	11.0	11.47
												11.47
3	30.50	17.02	19.40	17.02	8.0	17.95	16.61	39.5	1.63	1.71	3.8	6.935
	29.43	28.40	8.0	17.95	16.61	17.95	16.61	39.5	1.63	1.71	3.8	6.935
	25.26	25.264	31.530	14.86	14.816	18.404	6.70	6.889	23.464	1.70	1.618	14.37
												14.37
*MAS=Metabolic Activation System												
**CI=Confidence Interval												

Table 4. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCIG and TI Values for Glycerol with Coefficients of Variation.

Laboratory	Without MAS*										With MAS													
	LC50					EC50					MCIG					TI								
	Mean (mg/mL)		CV (%)	Mean (mg/mL)		CV (%)	Mean (mg/mL)		CV (%)	Mean (mg/mL)		CV (%)	Mean (mg/mL)		CV (%)	Mean (mg/mL)		CV (%)	Mean (mg/mL)		CV (%)			
	and 95% CI**			and 95% CI		and 95% CI		and 95% CI		and 95% CI		and 95% CI		and 95% CI		and 95% CI		and 95% CI						
1	17.03		11.1		15		1.53		11.4		10.58		10.00		1.08									
	10.32	14.84	21.5	10.21	10.89	4.5	15.00	1.01	1.35	17.8	6.24	10.33	29.0	11.26	3.3	10.00	8.67	21.8	0.55	0.97	31.8			
	17.16	10.41 - 19.26	11.35	10.21 - 11.56	11.35	10.21 - 11.56	8.00	8.09 - 17.24	1.51	1.02 - 1.68	13.34	6.18 - 14.48	10.44	10.26 - 11.25	10.76	6.00	6.05 - 11.28	1.28	0.54 - 1.40					
2	17.99		12.13		11.25		1.48		18.62		11.49		12.50		1.62									
	20.68	19.10	6.0	10.96	11.48	4.2	11.25	1.89	1.67	10.1	20.84	18.85	8.2	11.37	2.2	10.00	10.83	10.9	1.83	1.67	6.7			
	18.64	17.52 - 20.69	11.35	10.81 - 12.15	11.35	10.81 - 12.15	10.00	10.02 - 11.65	1.64	1.44 - 1.90	17.09	16.72 - 20.98	10.92	10.92 - 11.60	11.26	10.00	9.20 - 12.47	1.57	1.52 - 1.83					
3	10.00		7.50		3.63		1.33		14.75		6.88		3.63		2.15									
	8.25	9.42	8.8	5.75	6.67	10.8	3.63	3.63	0.0	1.43	1.41	4.4	11.00	13.46	12.9	5.50	5.84	12.9	1.38	2.13	49.8	2.00	2.33	15.9
	10.00	8.27 - 10.56	6.75	5.67 - 7.66	6.75	5.67 - 7.66	3.63	3.63	1.48	1.33 - 1.50	14.63	11.05 - 15.87	5.13	4.79 - 6.88	5.84	1.38	0.66 - 3.60	2.85	1.82 - 2.85					

MAS=Mercholic Activation System

*MAS=Metabolic Activation System

**CI=Confidence Interval

Table 6. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCIG and TI Values for Acrylamide with Coefficients of Variation.

Laboratory	Without MAS*						With MAS					
	LC50			EC50			LC50			EC50		
	Mean (mg/mL)	CV (%)	and 95% CI**	Mean (mg/mL)	CV (%)	and 95% CI	Mean (mg/mL)	CV (%)	and 95% CI	Mean (mg/mL)	CV (%)	and 95% CI
1	0.144	0.066	0.066	0.070	2.19	0.108	0.108	0.042	0.03	0.03	0.03	2.56
	0.166	5.9	0.066	0.063	7.5	0.050	0.081	0.102	15.3	0.032	33.8	0.03
	0.160	0.144 - 0.170	0.056 - 0.069	0.005	0.004	0.005	0.118	0.081	0.124	0.037	0.017	0.047
2	0.19	0.06	0.06	0.11	3.12	0.18	0.18	0.04	0.03	0.03	0.03	4.60
	0.18	45.0	0.05	0.08	8.3	0.08	0.18	0.240	35.4	0.047	20.2	0.08
	0.44	0.101 - 0.437	0.06 - 0.063	0.11	0.080 - 0.120	0.36	0.36	0.122 - 0.358	0.04	0.034	0.060	0.11
3	0.18	0.03	0.03	0.016	5.67	0.17	0.17	0.03	0.03	0.03	0.03	6.00
	0.25	13.8	0.05	0.040	20.4	0.015	0.19	0.167	12.3	0.037	12.9	0.015
	0.24	0.180 - 0.266	0.04 - 0.029	0.015	0.015	0.015	0.14	0.138	0.195	0.030	0.043	0.015
*MAS=Metabolic Activation System												
**CI=Confidence Interval												

Table 7. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCIG and TI Values for Triethylene Glycol Dimethylether with Coefficients of Variation.

Laboratory	Without MAS*										With MAS									
	LC50					EC50					MCIG					TI				
	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)
	and 95% CI**		and 95% CI		and 95% CI	and 95% CI		and 95% CI		and 95% CI	and 95% CI		and 95% CI		and 95% CI	and 95% CI		and 95% CI		and 95% CI
1	17.60	24.86	20.7	20.7	8.61	3.00	1.88	16.45	9.8	18.00	7.09	2.00	17.08	9.8	11.91	9.41	21.0	6.00	3.67	46.4
		28.30	17.74 - 31.98	9.20	7.29 - 9.92	3.00	2.04 - 5.96	3.08	1.79 - 4.15	20.48	15.55 - 20.46	9.22	6.67 - 12.14	3.00	1.31 - 6.02	2.22	1.44 - 2.54			
2	26.94	29.86	10.5	6.88	6.97	NA	3.33	21.25	39.5	14.56	6.58	NA	15.23	3.10	4.27	38.3	4.00	3.25	23.1	4.91
		34.23	25.50 - 34.22	5.89	5.70 - 8.24	4.00	1.75 - 13.75	5.81	2.99 - 5.86	7.20	6.58 - 22.54	3.13	2.01 - 6.53	2.50	2.05 - 4.45	2.30	1.98 - 4.98			
3	23.43	26.93	10.9	5.15	6.41	8.10	2.87	13.97	19.7	14.02	5.69	2.50	10.66	19.7	2.17	4.61	37.5	2.50	2.50	0.0
		30.60	22.87 - 30.99	5.90	4.63 - 8.19	8.10	8.10 - 8.10	5.19	2.90 - 5.94	17.43	10.19 - 17.85	5.96	2.21 - 7.00	2.50		2.92	1.96 - 4.90			

*MAS=Metabolic Activation System

**CI=Confidence Interval

Table 8. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCIG and TI Values for Diethylene Glycol with Coefficients of Variation.

Laboratory	Without MAS*								With MAS										
	LC50		EC50		MCIG		TI		LC50		EC50		MCIG		TI				
	Mean (mg/mL) and 95% CI**	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean and 95% CI	CV (%)			
1	30.58	21.08	15.00	1.45	23.77	18.84	20.00	1.26	23.77	18.84	20.00	1.26	23.77	18.84	20.00	1.26			
	28.90	30.03	2.7	16.94	18.74	9.3	20.00	13.33	46.8	1.71	1.61	7.2	21.51	23.81	8.0	15.89	13.41	42.7	20.00
	30.62	28.92 - 31.14	18.19	16.33 - 21.14	5.00	4.69 - 21.98	1.68	1.45 - 1.77	26.16	21.19 - 26.44	5.50	5.48 - 21.34	0.50	0.76 - 26.24	4.76	0.20 - 4.71	66.3	66.3	
2	43.00	17.20	19.00	2.50	34.36	14.39	11.00	2.39	34.36	14.39	11.00	2.39	34.36	14.39	11.00	2.39	34.36	14.39	11.00
	45.36	39.88	15.4	18.09	17.47	2.5	24.00	18.67	24.1	2.51	2.28	14.0	36.01	29.68	26.3	17.32	15.34	9.1	24.00
	31.29	31.36 - 48.41	17.11	16.85 - 18.08	13.00	12.43 - 24.90	1.83	1.84 - 2.72	18.67	18.85 - 40.51	14.31	13.40 - 17.28	19.00	10.58 - 25.42	1.30	1.29 - 2.56	23.8	23.8	
3	34.66	10.23	2.50	3.39	27.30	10.38	2.50	2.63	27.30	10.38	2.50	2.63	27.30	10.38	2.50	2.63	27.30	10.38	2.50
	30.72	32.48	5.0	6.84	9.74	22.5	5.10	3.37	36.4	4.49	3.50	21.8	24.34	26.33	5.3	6.65	8.65	17.7	2.50
	32.07	30.22 - 34.75	12.15	6.70 - 12.78	2.50	1.67 - 5.07	2.63	2.45 - 4.56	27.34	24.38 - 28.27	8.92	6.52 - 10.78	2.50	2.50	3.07	2.54 - 3.70	13.5	13.5	

*MAS=Metabolic Activation System

**CI=Confidence Interval

Table 9. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCIG and TI Values for Phthalic Acid with Coefficients of Variation.

Laboratory	Without MAS*										With MAS									
	LC50					EC50					MCIG					TI				
	Mean (mg/mL) and 95% CI**	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	NA	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	1.04	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	1.00	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	1.12
1	8.61		8.24			7.93		8.00			8.00		8.00			1.83		1.27		
	9.24	3.7	7.57	3.5	8.00	7.93	0.0	8.00	0.0	1.22	7.93	3.5	8.00	0.0	1.00	1.83	84.3	1.16	1.27	14.5
	8.49	8.33 - 9.23	7.98	7.54 - 8.31	8.00	7.54 - 8.31	7.1	8.00	7.1	1.07	7.98	6.22	8.00	6.22	0.50	-0.31 - 3.98	1.53	1.01	1.53	
2	8.89		8.68		NA	7.50		6.50		1.02	7.59		6.50		5.85	5.70		1.00		
	8.10	8.96	8.1	16.2	6.50	7.50	5.8	6.50	12.8	1.40	6.54	7.7	6.50	7.7	5.85	5.70	3.7	1.24	1.27	18.4
	9.88	7.95 - 9.97	7.99	5.82 - 9.18	7.30	5.82 - 9.18	1.24	7.30	1.44	1.24	7.84	4.99	7.30	4.99	5.40	5.41 - 5.99	1.57	0.95	1.59	
3	9.55		4.72		9.46	4.43		9.46		2.02	9.07		9.46		9.46	9.46		2.21		
	9.47	10.09	8.2	25.4	9.46	4.43	0.0	9.46	37.7	1.68	8.84	4.1	9.46	4.1	9.46	9.46	0.0	1.74	1.76	20.4
	11.26	8.95 - 11.24	2.93	2.87 - 5.99	9.46	2.87 - 5.99	3.84	9.46	383	3.84	9.73	7.32	9.46	7.32	9.46	9.46 - 9.46	1.33	1.26	2.26	

*MAS=Metabolic Activation System

**CI=Confidence Interval

Table 10. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCIG and TI Values for Dichloroacetate with Coefficients of Variation.

Laboratory	Without MAS*										With MAS										
	LC50					EC50					MCIG					TI					
	Mean (mg/mL)		CV	Mean (mg/mL)		CV	Mean (mg/mL)		CV	Mean (mg/mL)		CV	Mean (mg/mL)		CV	Mean (mg/mL)		CV	Mean (mg/mL)		CV
	and 95% CI**	(%)		and 95% CI	(%)		and 95% CI	(%)		and 95% CI	(%)		and 95% CI	(%)		and 95% CI	(%)		and 95% CI	(%)	
1	9.26		8.38		5.00		1.11														
	9.52	9.22	2.9	9.06	8.19	9.7	5.00	5.25	6.7	1.05	1.13	7.0	5.29	5.29	0.0	4.07	4.07	0.0	5.00	5.00	
	8.87	8.85 - 9.59		7.14	7.09 - 9.29		5.75	4.76 - 5.74		1.24	1.02 - 1.24										
2	6.70		2.34		2.80		2.86														
	6.42	6.57	1.7	2.11	1.88	26.4	6.25	3.08	80.4	3.04	3.81	32.0	6.20	6.30		0.90	0.92		1.65	7.00	
	6.58	6.41 - 6.73		1.19	1.19 - 2.57		0.20	-0.35 - 6.52		5.53	2.12 - 5.50		3.01	3.05 - 7.29		0.79	0.79 - 0.95		8.57	0.14 - 8.54	
3	6.50		4.28		5.83		1.52														
	6.92	7.03	6.8	4.90	5.15	16.1	5.83	6.51	14.8	1.41	1.38	9.0	6.59	6.51		3.94	4.77		5.83	1.65	
	7.66	6.36 - 7.69		6.27	4.00 - 6.30		7.88	5.17 - 7.85		1.22	1.21 - 1.56		6.71	6.49 - 6.72		4.28	3.86 - 4.80		7.88	5.17 - 7.85	

*MAS=Metabolic Activation System

**CI=Confidence Interval

Table 11. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCIG and TI Values for Sodium Bromate with Coefficients of Variation.

Laboratory	Without MAS*						With MAS					
	LC50			EC50			MCIG			TI		
	Mean (mg/mL) and 95% CI**	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean and 95% CI	CV (%)
1	1.34	0.28	0.10	0.25	0.20	0.10	0.22	0.62	0.05	0.07	0.36	0.36
	1.16	7.6	0.58	0.41	0.20	35.4	0.15	0.11	0.05	0.07	1.48	35.4
	1.13	1.08 - 1.33	0.36	0.23 - 0.58	0.25	0.10 - 0.30	0.15	0.08	0.10	0.03 - 0.10	1.80	1.80
2	1.05	0.32	0.50	0.16	0.27	58.6	0.75	0.29	0.21	0.19	2.59	12.2
	1.35	10.5	0.28	0.29	0.16	0.27	1.16	0.28	0.16	0.19	4.14	41.2
	1.15	1.01 - 1.36	0.27	0.26 - 0.32	0.16	0.05 - 0.50	0.69	0.24	0.21	0.16 - 0.23	2.88	2.88
3	2.12	0.49	1.30	0.39	1.19	13.5	0.72	0.20	0.57	0.39	3.60	36.0
	1.29	45.3	0.46	0.39	1.30	13.5	0.31	0.15	0.42	0.39	2.07	20.7
	0.63	0.50 - 2.19	0.21	0.21 - 0.56	0.96	0.96 - 1.41	0.18	0.11	0.18	0.17 - 0.61	1.64	1.64

*MAS=Metabolic Activation System

**CI=Confidence Interval

Table 12. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCI/G and TI Values for Tribromoacetic Acid with Coefficients of Variation.

Laboratory	Without MAS*						With MAS					
	LC50			EC50			MCI/G			TI		
	Mean (mg/mL)	CV (%)		Mean (mg/mL)	CV (%)		Mean (mg/mL)	CV (%)		Mean (mg/mL)	CV (%)	
	and 95% CI**			and 95% CI			and 95% CI			and 95% CI		
1	15.60	5.92	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
	14.58	15.59	5.3	9.27	7.97	18.4	7.97	18.4	7.97	18.4	7.97	18.4
	16.59	14.45 - 16.73	8.72	5.94	10.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
2	16.44	5.00	8.39	8.39	8.39	8.39	8.39	8.39	8.39	8.39	8.39	8.39
	17.90	15.87	12.1	5.06	4.27	25.3	4.27	25.3	4.27	25.3	4.27	25.3
	13.28	13.20 - 18.55	2.74	2.77	5.76	5.57	5.57	5.57	5.57	5.57	5.57	5.57
3	9.10	1.7	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09
	8.96	9.08	1.0	1.82	1.63	11.7	1.63	11.7	1.63	11.7	1.63	11.7
	9.19	8.95 - 9.21	1.37	1.37	1.89	6.09	6.09	6.09	6.09	6.09	6.09	6.09

*MAS=Metabolic Activation System

**CI=Confidence Interval

Table 13. Interlaboratory Summary Data with Coefficients of Variation

Chemical	Without MAS										With MAS									
	LC50					EC50					MCIG					TI				
	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	
Sodium Arsenite	0.104	19.1	0.098	27.6	0.066	34.9	1.09	16.6	0.046	28.1	0.035	26.7	0.066	73.1	1.35	22.2				
	0.088 - 0.119		0.069 - 0.126		0.063 - 0.070		0.86 - 1.32		0.031 - 0.061		0.026 - 0.044		0.021 - 0.112		1.15 - 1.54					
Boric Acid	0.978	29.2	0.348	43.9	0.239	60.3	3.38	54.7	0.589	43.9	0.266	42.4	0.254	61.5	2.39	50.2				
	0.665 - 1.291		0.165 - 0.531		0.062 - 0.417		0.86 - 5.91		0.375 - 0.804		0.128 - 0.404		0.068 - 0.440		1.50 - 3.28					
Ethylene Glycol	26.55	19.1	13.21	20.2	13.60	30.6	2.10	31.2	25.30	26.0	12.49	30.6	11.36	46.3	2.30	47.7				
	20.21 - 32.89		9.68 - 16.75		10.70 - 16.51		1.24 - 2.95		17.76 - 32.84		7.57 - 17.40		7.79 - 14.93		0.83 - 3.76					
Glycerol	14.45	30.8	9.68	22.9	9.04	48.1	1.48	15.0	14.21	29.2	9.28	26.9	7.21	55.0	1.66	37.7				
	8.96 - 19.95		6.71 - 12.65		3.64 - 14.45		1.29 - 1.67		9.33 - 19.09		5.89 - 12.68		2.08 - 12.34		0.89 - 2.43					
Sodium Iodoacetate	0.386	69.4	0.413	70.3	0.483	56.5	1.24	87.2	0.183	114.0	0.421	166.6	0.207	2.3	0.99	111.5				
	0.099 - 0.672		0.189 - 0.636		-0.135 - 1.100		-0.14 - 2.61		-0.073 - 0.438		-0.035 - 0.876		NA - NA		-0.40 - 2.38					
Acrylamide	0.216	39.6	0.053	21.4	0.052	75.6	4.25	40.1	0.170	44.8	0.038	27.7	0.042	74.8	4.60	40.7				
	0.152 - 0.280		0.040 - 0.066		0.003 - 0.101		2.47 - 6.03		0.092 - 0.248		0.030 - 0.047		0.008 - 0.075		3.48 - 5.72					
Triethylene Glycol Dimethylether	27.22	16.1	7.33	19.3	6.48	43.6	3.94	30.7	15.53	27.1	6.09	48.3	3.13	39.0	2.97	38.4				
	24.38 - 30.06		6.03 - 8.62		3.90 - 9.05		2.99 - 4.89		13.08 - 17.97		2.84 - 9.35		2.46 - 3.79		2.01 - 3.92					
Diethylene Glycol	34.13	16.4	15.31	28.1	11.79	65.9	2.47	37.3	26.61	19.9	12.47	36.1	11.33	79.0	2.50	44.8				
	28.33 - 39.94		9.80 - 20.82		3.00 - 20.58		1.38 - 3.55		23.28 - 29.94		8.57 - 16.36		2.31 - 20.36		1.82 - 3.18					
Phthalic Acid	9.28	9.5	6.62	27.7	8.31	13.2	1.61	52.4	6.82	37.5	4.93	39.9	5.66	57.2	1.43	24.8				
	8.47 - 10.08		4.46 - 8.78		6.86 - 9.76		0.73 - 2.50		3.80 - 9.85		3.35 - 6.51		1.35 - 9.98		1.11 - 1.75					
Dichloroacetate	7.60	15.8	5.07	52.8	4.95	42.4	2.11	66.4	5.80	21.0	2.81	60.4	5.37	43.2	3.35	70.6				
	6.00 - 9.21		1.50 - 8.65		2.99 - 6.91		0.44 - 3.78		4.90 - 6.70		0.63 - 4.99		4.10 - 6.63		0.45 - 6.25					
Sodium Bromate	1.25	29.7	0.36	32.1	0.55	84.8	3.59	25.5	0.48	70.4	0.23	67.2	0.22	75.4	2.28	47.7				
	1.15 - 1.35		0.29 - 0.43		-0.07 - 1.18		3.07 - 4.11		0.08 - 0.88		0.15 - 0.31		0.03 - 0.40		1.15 - 3.42					
Tribromoacetic acid	13.52	24.9	4.62	60.7	6.18	20.4	3.86	41.8	9.36	41.2	3.84	81.3	4.47	71.5	4.14	80.0				
	9.17 - 17.86		1.02 - 8.23		4.79 - 7.57		1.81 - 5.91		7.08 - 11.65		0.30 - 7.38		3.36 - 5.59		0.63 - 7.66					

Table 14. Estimation of Teratogenic Hazard for Each Chemical¹.

Chemical	Criteria #1		Criteria #2	Criteria #3	Consensus
	TI ² w/o MAS ³	TI w MAS	Severity w MAS	MAS MCIG < 30% of LC50	
Sodium Arsenite	N ⁴	N	N	N	N
Boric Acid	N	T	E	N	E
Ethylene Glycol	N	N	E	N	N
Glycerol	N	N	N	N	N
Sodium iodoacetate	N	N	N	N	N
Acrylamide	T	T	T	T	T
Triethylene glycol dimethylether	T	T	T	T	T
Diethylene glycol	N	T	E	N	T
Phthalic Acid	N	N	N	N	N
Dichloroacetate	N	N	N	N	N
Sodium Bromate	T	N	T	N	E
Tribromoacetic acid	T	N	E	N	N

¹ The TI w/o MAS column not used in determining consensus.

² TI=Teratogenic Index (96-hr LC50/96-hr EC50(malformation)).

³ MAS=Metabolic Activation System consisting of Aroclor 1254-induced rat liver microsomes.

⁴ N=Nonteratogen; E=Equivocal Teratogen; T=Teratogen.

Table 15. Interlaboratory Endpoint Variability Based on Coefficient of Variation Values.

Chemical	Endpoint	Variability	No MAS ¹	Endpoint	Variability	MAS
	Least	Mid	Most	Least	Mid	Most
Sodium Arsenite	LC50	EC50	MCIG	EC50	LC50	MCIG
Boric Acid	LC50	EC50	MCIG	EC50	LC50	MCIG
Ethylene Glycol	LC50	EC50	MCIG	LC50	EC50	MCIG
Glycerol	EC50	LC50	MCIG	EC50	LC50	MCIG
Sodium Iodoacetate ²	MCIG	LC50	EC50	LC50	EC50	
Acrylamide	EC50	LC50	MCIG	EC50	LC50	MCIG
Triethylene Glycol	LC50	EC50	MCIG	LC50	MCIG	EC50
Dimethyl-ether						
Diethylene Glycol	LC50	EC50	MCIG	LC50	EC50	MCIG
Phthalic Acid	LC50	MCIG	EC50	LC50	EC50	MCIG
Dichloroacetate	LC50	MCIG	EC50	LC50	MCIG	EC50
Sodium bromide	LC50	EC50	MCIG	EC50	LC50	MCIG
Tribromoacetic acid	MCIG	LC50	EC50	LC50	MCIG	EC50

¹ MAS=Metabolic Activation System.

² MAS MCIG could not be calculated.

Table 16. FETAX Teratogenic Values Recorded During Validation.

Range of Teratogenic Values	Number of Historical TI Values in Validation Study (n)	Number of TI Values in Phase III-Part3 (n)
<1	3	1
1-1.5	32	5
1.6-2.0	13	2
2.1-2.5	14	7
2.6-3.0	4	1
3.1-3.5	5	3
3.6-4.0	5	2
4.1-4.5	7	2
4.6-5.0	1	1
5.1-5.5	1	
5.6-6.0	2	
6.1-6.5	1	
6.6-7.0	0	
7.1-7.5	1	
7.6-8.0	1	
8.1-8.5	0	
8.6-9.0	0	
9.1-9.5	0	
9.6-10.0	0	
10.1-20.0	11	
20.1-30.0	1	
30.1-40.0	1	
40.1-50.0	0	
50.1-60.0	1	
60.1-70.0	0	
70.1-80.0	0	
80.1-90.0	0	
90.1-100.0	0	
100.1-150.0	1	
150.1-200.0	0	
200.1-250.0	1	
250.1-300.0	0	
300.1-350.0	1	
350.1-400.0	0	
400.1-450.0	1	
450.1-500.0	0	
500.1-550.0	0	
550.1-600.0	0	
>600	1	
Total TI Values Recorded	109	24

INTERLABORATORY STUDY OF FETAX

PHASE III, PART 3

SODIUM ARSENITE
DATA SUMMARY SHEETS
PHASE III-PART 3

060111-0115 NB 11 775
 FINCH. #4
 Arsenite w/o MAS #1

FETAX Summary Sheet

Test No. 206-009 I

Test Material -	NA w/o MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABROL
CAS No.	84	Lot No.	E5
Composition/Purity	08	Test Start Date:	24 OCT 94
Solvent	87	Test End Date	28 OCT 94
Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7.8	7.7	7.9	7	
Control		6.9	6.8	7	6.8
Highest Concentration		7.7	7.4	8	7.3

No. Dead or Malformed		
X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	0 : 80 X 100 = 0%	3 : 80 X 100 = 3.8%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm)	10.344	Solvent Control Length (mm) J20
Minimum Concentration to Inhibit Growth (MCIG):	0.06	MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.1	0.06	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.106	EC50	0.100
95% CL	0.099 — 0.112	95% Confidence limits	0.096 — *****
Test Teratogenic Index (TI = LC50/EC50):		1.05	
95% Confidence limits		0.97 — 1.13	

FINCH NB1177 J
 Arsenite w MAS #1

FETAX Summary Sheet

Test No. 206-009 A

Test Material	NA W/ MAS	Investigator	DR. FINCH
Source	OSU -	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	08	Test Start Date:	24 OCT 94
Solvent	B7	Test End Date	28 OCT 94
	Conc.	E7	Test Units (i.e., mg/ml)
			MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.8	7.7	7.9	7.3	
Control		6.9	6.8	6.8	6.8
Highest Concentration		7	7	7	6.8

No. Dead or Malformed				
X 100 = %				
Total Number	Mortality Record		Malformation Record	
FETAX Control	2 : 80	X 100 = 0.025	0 : 78	X 100 = 0
Solvent Control	:	X 100 =	:	X 100 =
Control Length (mm)	9.841	Solvent Control Length (mm)	J20	
Minimum Concentration to Inhibit Growth (MCIG)	0.04			MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.04	N.A.	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.0632043	EC50	0.037714
95% CL	0.053052	95% Confidence limits	0.0263 ---- 0.054
Test Teratogenic Index (TI = LC50/EC50):			1.6229
95% Confidence limits			1.100027 -- 2.39417

FINCH NB177 J
Aroclor w/o MAS #2

FETAX Summary Sheet

Test No. 206-012 I

Test Material	NA w/o MAS	Investigator	DR. FINCH
Source	OSU-	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Test Start Date:	12 DEC 94	Test End Date	16 DEC 94
Composition/Purity	08	Test Units (i.e., mg/ml)	MG/ML
Solvent	B7	Conc.	E7

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7.7	7.8	7.4	7.8	
Control		6.9	6.9	6.9	6.9
Highest Concentration		7.3	7.4	7.2	7.3

No. Dead or Malformed		
X 100 = %		
Total Number		
FETAX Control	Mortality Record	Malformation Record
Solvent Control	1 : 80 X 100 = 0.0125	2 : 79 X 100 = 0.025
Control Length (mm)	10.444	Solvent Control Length (mm) J20
Minimum Concentration to Inhibit Growth (MCIG)	0.06	MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.06	0.04	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.0873875	EC50	0.09394
95% CL	0.0824105 - 0.0927	95% Confidence limits	0.1366 - 0.065
Test Teratogenic Index (TI = LC50/EC50):		0.9303	
95% Confidence limits		0.636844 - 1.35884	

FETAX Summary Sheet

Test No. 206-012 A

Test Material	NA W/ MAS	Investigator	DR. FINCH
Source	OSU-	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	08	Test Start Date:	12 DEC 94
Solvent	B7	Test End Date	16 DEC 94
Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.7	7.8	7.4	7.8	
Control	6.9	6.9	6.9	6.9	
Highest Concentration	7.5	7.3	ND	ND	

No. Dead or Malformed				
X 100 = %				
Total Number	Mortality Record		Malformation Record	
FETAX Control	0 : 80	X 100 = 0	4 : 80	X 100 = 0.05
Solvent Control	:	X 100 =	:	X 100 =
Control Length (mm)	9.625	Solvent Control Length (mm)	J20	
Minimum Concentration to Inhibit Growth (MCIG)	(0.04)			MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	N.A.	0.06	T-test
LOEL	N.A.	N.A.	T-test
LC50	(0.0419)729	EC50 (0.0497)02	
95% CL	0.0356819 -- 0.0494	95% Confidence limits 0.0437 --- 0.057	
Test Teratogenic Index (TI = LC50/EC50): (0.84)5			
95% Confidence limits 0.686225 -- 1.03926			

FINCH
Arsenite #w/o MAS # 5

FETAX Summary Sheet

Test No. 206-013 I

Test Material	NA w/o MAS	Investigator	DR. FINCH
Source	OSU -	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C8	Test Start Date:	09 JAN 95
Solvent	B7	Test End Date	13 JAN 95
Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.6	7.6	7.8	7.8	
Control		6.9	6.8	6.9	6.9
Highest Concentration		7.1	7.3	7.5	7.6

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length (mm)

9.117

Solvent Control Length (mm)

J20

Minimum Concentration to Inhibit Growth (MCIG)

0.08

MG/ML

Mortality Record

Malformation Record

0 : 80

X 100 =

0%

2 : 80

X 100 =

0.025

X 100 =

X 100 =

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	N.A.	0.06	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.0737	306	EC50 0.0685462
95% CL	0.05562558	-- 0.0977	95% Confidence limits 0.0617 --- 0.076

Test Teratogenic Index (TI = LC50/EC50):

1.0754

95% Confidence limits

0.80

--

1.45

FINCH NB1177 J
Aroclite w MAS #3

FETAX Summary Sheet

Test No. 206-013 A

Test Material	NA w/MAS	Investigator	Dr. Finch
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	08	Test Start Date:	09 JAN 95
Solvent	B7	Test End Date	13 JAN 95
Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7.6	7.6	7.8	7.8	
Control		6.9	6.8	6.9	6.9
Highest Concentration		7	7	7.1	7.1

No. Dead or Malformed				
X 100 = %				
Total Number				
FETAX Control				
Solvent Control				
Control Length (mm)	9.115	Solvent Control Length (mm)	J20	
Minimum Concentration to Inhibit Growth (MCIG)	0.04		MG/M	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.04	0.04	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.06101061	EC50	0.041783
95% CL	0.05561638	95% Confidence limits	0.0351 --- 0.05

Test Teratogenic Index (TI = LC50/EC50):	1.4602
95% Confidence limits	1.20 -- 1.78

Chemical Code:	NA	Test No.:	7
Compound:	Sodium Arsenite	Investigator:	Mendi A. Hull
Source:	Sigma	Laboratory:	OSU / Bantle
CAS No.:	79-43-6	Start Date:	3-Apr-96
Lot No.:	25H3432	End Date:	7-Apr-96
Glass / Plastic	Plastic	Test Units:	mg/mL
Microsome lot No.:	M18		

	% Mortality	% Malformation
FETAX AB Controls	0	7.4
FETAX Controls	0	10.0
MAS Controls	0	5.0

Cyclophosphamide

Positive Control	100	—
Negative Control	0	5

Results

Without the Metabolic Activation System

LC50	0.090	EC50	0.072
95% CI	0.081 - 0.10	95% CI	0.052 - 0.099
Control length	0.92397 cm	TI	1.25
MCIG	0.065 mg/mL	95% CI	

With the Metabolic Activation System

LC50	0.052	EC50	0.033
95% CI	0.048 - 0.056	95% CI	0.020 - 0.055
Control length	0.97511 cm	TI	1.58
MCIG	0.034 mg/mL	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

Chemical Code:	NA	Test No.:	8
Compound:	Sodium Arsenite	Investigator:	Mendi A. Hull
Source:	Sigma	Laboratory:	OSU / Bantle
CAS No.:	79-43-6	Start Date:	3-Apr-96
Lot No.:	25H3432	End Date:	7-Apr-96
Glass / Plastic	Plastic	Test Units:	mg/mL
Microsome lot No.:	M18		

	% Mortality	% Malformation
FETAX AB Controls	2.5	10.1
FETAX Controls	1.25	10.0
MAS Controls	5.0	7.9

Cyclophosphamide

Positive Control	100	—
Negative Control	0	5

Results

Without the Metabolic Activation System

LC50	0.102	EC50	0.077
95% CI	0.086 - 0.121	95% CI	0.0691 - 0.0853
Control length	0.95041 cm	TI	1.32
MCIG	0.065 mg/mL	95% CI	

With the Metabolic Activation System

LC50	0.0531	EC50	0.0394
95% CI	0.0495 - 0.0576	95% CI	0.0149 - 0.1044
Control length	0.86305	TI	1.36
MCIG	none	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

Arsenite w/w/o MAS #3

Chemical Code:	NA		
Compound:	Sodium Arsenite	Test No.:	9
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:	79-43-6	Laboratory:	OSU / Bantle
Lot No.:	25H3432	Start Date:	16-Apr-95 <i>96</i> ^{HA}
Glass / Plastic	Plastic	End Date:	20-Apr-95 <i>96</i> ^{HA}
Microsome lot No.:	M20	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	0	1.2
FETAX Controls	1.25	2.5
MAS Controls	0	5.0

Cyclophosphamide

Positive Control	100	—
Negative Control	0	10

Results

Without the Metabolic Activation System

LC50	0.124	EC50	0.089
95% CI	0.075 - 0.205	95% CI	0.079 - 0.099
Control length	0.90104 cm	TI	1.39
MCIG	0.078 mg/mL	95% CI	

With the Metabolic Activation System

LC50	0.053	EC50	0.032
95% CI	0.041 - 0.069	95% CI	0.012 - 0.081
Control length	0.84850 cm	TI	1.66
MCIG	0.047 mg/mL	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

FETAX -- U.S. ARMY ILS PROJECT (OKLA01 - RSTS01) RESULTS WITH NA

ENDPOINTS*					
TEST	NO.	MAS	LC50	EC50	TI
Range	R	N	0.09	>0.1	~1.0
Unactivated	1	N	0.10	0.09	1.11
			(0.09-0.11)	(0.085-0.095)	
Activated** FORT 1	1	Y	0.02/0.09	0.02/0.09	1.0/1.0
			(0.01-0.02)/(0.08-0.11)	(0.01-0.02)/(0.08-0.11)	
FORT 2	2	Y	0.03/0.014	0.02/0.14	1.6/1.0
			(0.03-0.034)/(0.13-0.15)	(0.01-0.03)/(0.13-0.15)	
FORT 3	3	Y	0.04/0.12	0.04/>0.15	1.0/0.8
			(0.03-0.05)/(0.11-0.12)	(0.03-0.05)/(NA)	
					0.03/0.09

20097
Checked 7/17/96
YMT

* Expressed as mg/mL.
** Activated/Unactivated

FETAX SUMMARY SHEET

FORT
Arsenite w w/o MAS # 1

Test No. 2

Test Material	NA	Investigator	
Source		Lab	
CAS No.		Lot No.	
Composition/Purity		Test Start Date	5/23/94
Solvent		Test End Date	5/27/94
		Test Units (i.e., mg/ml)	

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	11.0/8.0	11.5/8.0	11.0/7.5	11.3/8.3	
Control	8.0	8.0	8.1	8.1	
Highest Conc.	7.8	8.1	8.0	8.0	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %	0 : 80 X 100 = 0 %	3 : 80 X 100 = 4 %
Solvent Control	0 : 40 X 100 = 0 %	0 : 40 X 100 = 0 %
Control Length 779 mm MAS 66.7	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	0.09	w/o MAS - 0.15

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	failed var.	failed var.	
LOEL	" "	" "	
LC ₅₀ MAS : 0.09		EC ₅₀ MAS : 0.09	
95% Confidence limits 0.01 - 0.6 MAS		95% Confidence Limits 0.01 - 0.6 MAS	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀)

1.0

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 = ___ %

FORT
Arsenite ~~#2~~ w/ w/o MAS #2 L

FETAX SUMMARY SHEET

Test No. 4

Test Material	NA	Investigator	Fort
Source	TLS	Lab	SBL
CAS No.		Lot No.	
Composition/Purity		Test Start Date	8/3/94
Solvent		Conc.	
		Test End Date	8/7/94
		Test Units (i.e., mg/mL)	mg/mL

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.2	7.5	7.6	7.8	
Control	7.8	7.9	7.9	7.8	
Highest Conc.	7.5	7.5	7.6	7.7	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %		
MAS	0 : 80 X 100 = 0 %	1 : 80 X 100 = 1.3 %
Solvent Control	0 : 40 X 100 = 0 %	2 : 40 X 100 = 5 %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

w/o MAS

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	ND	ND	Failed Assumption
LOEL	ND	ND	Failed Assumption
LC ₅₀ ^{W/MAS}	0.14 / 0.032	EC ₅₀	0.14 / 0.02
95% Confidence Limits	(0.13-0.15) (0.03-0.032)	95% Confidence Limits	(0.13-0.15) (0.01-0.03)
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			
1.9 / 1.6			

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 = ___ %

Arsenide/w/omas

#3

FETAX SUMMARY SHEET

Test No. DEF-2

Test Material	NA	Investigator	M. JACKSON
Source		Lab	STOVER/FRT
CAS No.		Test Start Date	7/12/94
Lot No.		Test End Date	7/16/94
Composition/Purity		Test Units (l.o., mg/L)	mg/L
Solvent			

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	9.0/8.5	9.1/8.8	9.0/8.5	9.0/8.5	
Control	8.0	7.8	7.8	7.8	
Highest Conc.	8.4	8.4	8.4	8.4	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead of Replicates		
% 100 = %		
Total Number		
NA	0: 80 X 100 = 0%	0: 80 X 100 = 0%
Solvent Control	0: 40 X 100 = 0%	0: 40 X 100 = 0%
Control Length 90.2 mm	Solvent Control Length 84.9 mm	
Minimum Concentration to Inhibit Growth (MICG)	0.009	MAS = 0.03

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.09	0.009	TOXSTAT
LOEL	0.15	0.03	TOXSTAT
LC ₅₀	0.12	0.04	EC ₅₀
95% Confidence Limits	0.03-0.05	0.03-0.05	
TEST TEST INDEX (TI = LC ₅₀ / EC ₅₀)			
0.8			

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L		
2500 mg/L		

BORIC ACID
DATA SUMMARY SHEETS
PHASE III-PART 3

Copied 10-95
 NB1177 J
 FINCH
 Boric Acid w/o MAS #1

FETAX Summary Sheet

Test No. 207-003 I

Test Material -	NB w/o MAS	Investigator	DR.FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	34	Lot No.	E5
Composition/Purity	08	Test Start Date:	06 FEB 95
Solvent	37	Test End Date	10 FEB 95
Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
Stack	7.5	7.4	7.5	7	
Control		6.9	7	7	6.9
Highest Concentration		7.7	7.3	8	7.2

No. Dead or Malformed					
X 100 = %					
Total Number	Mortality Record		Malformation Record		
	FETAX Control		FETAX Control		
	Solvent Control		Solvent Control		
Control Length (mm)	10.073	Solvent Control Length (mm)		J20	
Minimum Concentration to Inhibit Growth (MCIG)	0.4				MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.8	0.5	T-test
LOEL	N.A.	N.A.	T-test
LC50	1.365	EC50	0.548
95% CL	1.608	95% Confidence limits	0.504 ----- *****
Test Teratogenic Index (TI = LC50/EC50):			2.49
95% Confidence limits			2.07 ----- 2.99

NB1177 J
Boric Acid w. MAS #1

FETAX Summary Sheet

Test No. 207-003 A

Test Material -	NB w/MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USASROD
CAS No.	84	Lot No.	E5
Test Start Date:	06 FEB 95	Test End Date	10 FEB 95
Composition/Purity	08	Test Units (i.e., mg/ml)	MG/ML
Solvent	B7	Conc.	E7

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.5	7.4	7.5	7	
Control		6.9	7	6.8	6.9
Highest Concentration		7.6	7.6	7.3	7.2

No. Dead or Malformed
X 100 = %
Total Number
FETAX Control
Solvent Control

MALFORMATION EXCEED ASTM LIMITS

Mortality Record		Malformation Record	
0 : 80	X 100 = 0%	8 : 80	X 100 = 10.0%
:	X 100 =	:	X 100 =
Control Length (mm)	9.475	Solvent Control Length (mm)	J20
Minimum Concentration to Inhibit Growth (MCIG)		0.4	MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.9	0.4	T-test
LOEL	N.A.	N.A.	T-test
LC50	1.011	EC50	0.442
95% CL	0.949 — *****	95% Confidence limits	0.382 — *****
Test Teratogenic Index (TI = LC50/EC50):		2.29	
95% Confidence limits		1.95 —	2.68

FINCH (5-10-95) SUBMITT J
 Boie acid WYOMAS #2

FETAX Summary Sheet

Test No. 207-004 I

Test Material	NB W/O MAS		Investigator	DR. FINCH
Source	OSU		Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date: 06 FEB 95
Composition/Purity	C6		Test End Date	10 FEB 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml) MG/ML

		Day 0	Day 1	Day 2	Day 3	Day 4
pH	Stock	7.5	7.4	7.5	7	
	Control		6.9	7	6.8	6.9
	Highest Concentration		7.7	7.3	7.6	7.2

No. Dead or Malformed			MALFORMATION EXCEED ASTM	
X 100 = %				
Total Number			Mortality Record	Malformation Record
FETAX Control	1 : 80	X 100 = 1%	10 : 79	X 100 = 12.7%
Solvent Control	:	X 100 =	:	X 100 =
Control Length (mm)	9.815		Solvent Control Length (mm)	J20
Minimum Concentration to Inhibit Growth (MCIG)	0.4		MG/M	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.7	0.5	T-test
LOEL	N.A.	N.A.	T-test
LC50	1.010	EC50	0.462
95% CL	0.726 -- 1.406	95% Confidence limits	0.383 ---- 0.557
Test Teratogenic Index (TI = LC50/EC50):		2.19	
95% Confidence limits		1.50 --	3.20

FINCH NB11775
BORIC ACID W MAS #2

FETAX Summary Sheet

Test No. 207-004 A

Test Material	NB W/MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Test Start Date:	6 FEB 95	Test End Date	10 FEB 95
Composition/Purity	OS	Test Units (i.e., mg/ml)	MG/ML
Solvent	B7	Conc.	E7

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7.5	7.4	7.5	7	
Control		6.9	7	6.8	6.9
Highest Concentration		7.7	7.5	7.4	7.2

No. Dead or Malformed					MALFORMATION EXCEED ASTM L	
X 100 = %						
Total Number	Mortality Record		Malformation Record			
FETAX Control	0 : 80	X 100 = 0%	12 : 80	X 100 = 15.0%		
Solvent Control	:	X 100 =	:	X 100 =		
Control Length (mm)	9.556	Solvent Control Length (mm)		J20		
Minimum Concentration to Inhibit Growth (MCIG)	0.4			MG/M		

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.8	0.5	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.870	EC50	0.466
95% CL	0.786	95% Confidence limits	0.395 ---- 0.549
Test Teratogenic Index (TI = LC50/EC50):		1.87	
95% Confidence limits		1.54	2.27

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FINCH

BORIC ACID w/o MAS #

FETAX Summary Sheet

Test Material - NB w/o MAS				Investigator DR. FINCH	
Source OSU				Laboratory USABRDL	
CAS No. B4	Lot No. E5		Test Start Date: 27 FEB 95		
Composition/Purity 08			Test End Date 03 MAR 95		
Solvent B7	Conc. E7		Test Units (i.e., mg/ml) MG/ML		

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.05	6.9	6.8	7.05	
Control		7	7.4	6.9	6.9
Highest Concentration		7.3	7.4	7.1	7.2

No. Dead or Malformed X 100 = % Total Number FETAX Control Solvent Control	Mortality Record		Malformation Record	
	5 : 80	X 100 = 6%	4 : 75	X 100 = 5.3%
	:	X 100 =	:	X 100 =
	Control Length (mm) 9.832		Solvent Control Length (mm) J20	
Minimum Concentration to Inhibit Growth (MCIG) 0.4 MG/ML				

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	1	0.5	T-test
LOEL	N.A.	N.A.	T-test
LC50	1.110	EC50 0.525	
95% CL	1.072 — 1.149	95% Confidence limits 0.479 — ****	
Test Teratogenic Index (TI = LC50/EC50): 2.11			
95% Confidence limits 1.92 — 2.33			

BANTLE
BORIC ACID w/o MAS #1

FETAX Summary Sheet

Test No. 1a

Test Material	NB	Investigator	Mendi A. Hull
Source		Laboratory	OSU Bantle
CAS No.	Lot No.	Test Start Date:	14 July 1994
Composition/Purity		Test End Date	18 July 1994
Solvent	Conc.	Test Units (i.e., mg/ml)	mg/ml

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7	7	7	7	
Control		7.4	7.4	7.6	7.5
Highest Concentration		7.6	7.6	7.6	7.5

No. Dead or Malformed		
X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	0 : 80 X 100 = 0%	8 : 80 X 100 = 10.0%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm) C20	Solvent Control Length (mm) J20	
Minimum Concentration to Inhibit Growth (MCIG) G21	mg/ml	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.36	0.18	T-test
LOEL	0.74	0.27	T-test
LC50	1.217	EC50	0.226
95% CL	0.888 -- 1.667	95% Confidence limits	0.183 ---- 0.279
Test Teratogenic Index (TI = LC50/EC50):			5.39
95% Confidence limits			3.69 -- 7.88

Percent effect	LC	EC
5	0.5928365	0.098
16	0.7877523	0.136
50	1.2166319	0.226
84	1.8790086	0.375
95	2.4967984	0.522

BANTLE
BORIC ACID W MAS #1

FETAX Summary Sheet

Test No. 1b	
Test Material HB	Investigator Mendi A. Hull
Source	Laboratory OSU Bantle
CAS No. Lot No.	Test Start Date: 14 July 1994
Composition/Purity	Test End Date 18 July 1994
Solvent Conc.	Test Units (i.e., mg/ml) mg/ml

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7	7	7	7	
Control		7.4	7.4	7.6	7.5
Highest Concentration		7.6	7.6	7.6	7.5

No. Dead or Malformed $\times 100 = \%$ Total Number	Mortality Record		Malformation Record	
	FETAX Control	0 : 80 $\times 100 = 0\%$	8 : 80 $\times 100 = 10.0\%$	
	Solvent Control	: $\times 100 =$	: $\times 100 =$	
Control Length (mm) C20		Solvent Control Length (mm) J20		
Minimum Concentration to Inhibit Growth (MCIG) G21				mg/ml

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used	
NOEL	0.36	0.08	T-test	
LOEL	0.74	0.11	T-test	
LC50	0.438	EC50	0.127	
95% CL	0.365	--	0.526	95% Confidence limits 0.101 ---- 0.161
Test Teratogenic Index (TI = LC50/EC50):			3.44	
95% Confidence limits			2.55	-- 4.63

Percent effect	LC	EC
5	0.1902336	0.058
16	0.2645562	0.079
50	0.4380525	0.127
84	0.7253277	0.206
95	1.0087067	0.282

BANTLE
BORIC ACID W W/O MAS #2

MAS range summary sheets.

FETAX Summary Sheet

Test No. Definitive 1

Test Material	NB	Investigator	MENDI HULL
Source	SIGMA	Laboratory	BANTLE
CAS No.	Lot No.	Test Start Date:	18 July 1994
Composition/Purity		Test End Date	22 July 1994
Solvent	Conc.	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7.2	7.2	7.2	7.2	
Control		7.5	7.5	7.6	7.6
Highest Concentration		7.5	7.5	7.5	7.5

No. Dead or Malformed		
X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	2 : 80 X 100 = 2.5%	6 : 78 X 100 = 7.7%
Mas Control	0 : 40 X 100 = 0.0%	1 : 40 X 100 = 2.5%
Control Length (mm)	0.92783	MAS Control Length (mm) 0.89649
Minimum Concentration to Inhibit Growth (MCIG)	0.1	MG/ML

MAS MCIG = (0.1) mg/ml

Test Material/Compound Results

LC50	1.075	EC50	0.166
95% CL	0.955 -- 1.210	95% Confidence limits	0.141 ---- 0.196
Test Teratogenic Index (TI = LC50/EC50):		6.48	
95% Confidence limits		5.29 -- 7.94	

Test material/compound Results with MAS

LC50	0.900	EC50	0.169
95% CL	0.795 -- 1.019	95% Confidence limits	0.147 ---- 0.193
Test Teratogenic Index (TI = LC50/EC50):		5.34	
95% Confidence limits		4.44 -- 6.42	

Comparison Results	mas:no mas	no mas : mas
LC50	1.194	0.83752
EC50	0.984	1.01626
TI	1.2134	0.82412

MAS RANGE
FETAX Summary Sheet

BANTLE
BORIC ACID W WLOMAS #3

Test Material NB		Investigator Mendi A. Hull	
Source		Laboratory OSU/Bantle	
CAS No.	Lot No.	Test Start Date: 7 Sept 1994	
Composition/Purity		Test End Date 11 Sept 1994	
Solvent	Conc.	Test Units (i.e., mg/ml) mg/ml	
Embryos per Dish = 20			
Dish type: PLASTIC			

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7.4	7.4	7.4	7.4	
Control		7.6	7.55	7.6	7.55
Highest Concentration		7.55	7.5	7.5	7.55

No. Dead or Malformed		
X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	0 : 80 X 100 = 0.0%	6 : 80 X 100 = 7.5%
Mas Control	0 : 40 X 100 = 0.0%	3 : 40 X 100 = 7.5%
Control Length (mm)	MAS Control Length (mm)	
Minimum Concentration to Inhibit Growth (MCIG) ? 0.1 / ? mg/ml		

Test Material/Compound Results

LC50	1.046	EC50	0.175
95% CL	0.935 -- 1.169	95% Confidence limits	0.145 ---- 0.211
Test Teratogenic Index (TI = LC50/EC50):		5.98	
95% Confidence limits		4.80 -- 7.45	

Test material/compound Results with MAS

LC50	0.188	EC50	0.170
95% CL	0.116 -- 0.307	95% Confidence limits	0.143 ---- 0.201
Test Teratogenic Index (TI = LC50/EC50):		1.11	
95% Confidence limits		0.66 -- 1.86	

Comparison Results	Activated	Unactivated
LC50 - RATIO	5.55	0.18
95% Confidence limit	3.36 9.16	0.11 0.30
EC50 - RATIO	1.03	0.97
95% Confidence limits	0.80 1.33	0.75 1.25
TI - RATIO	0.19	5.39
95% Confidence limits	0.11 0.33	3.08 9.46

FETAX - U.S. ARMY ILS PROJECT (OKLA01-RSTS01) RESULTS WITH NB

TEST	NO.	MAS	ENDPOINTS*				MCIG
			LC50	EC50	TI		
Range	R	N	0.75	0.15**	5.00		0.1
Unactivated	1	N	0.66	0.67	0.99		0.5
Activated*** FORT 1		Y	0.52/1.01 (0.29-0.75)/(0.75-1.26)	>0.29/0.52 (-)/(0.29-0.75)	<1.79/1.94		0.015/ ~~0.15~~
FORT 2		Y	0.40/0.42 (0.37-0.43)/(0.40-0.45)	0.25/0.27 (0.24-0.27)/(0.26-0.28)	1.60/1.56		0.15(0.25)
FORT 3		Y	0.44/0.55 (0.41-0.46)/(0.49-0.61)	0.20/0.24 (0.18-0.22)/(0.21-0.27)	2.20/2.29		0.24/0.25

* Expressed as mg/mL.

** Results were biased by range selected.

*** Activated/Unactivated

FORT
BORIC ACID W W/MAS #11 DF

FETAX SUMMARY SHEET

Test No. 2

Test Material	NB	Investigator	
Source		Lab	
CAS No.	Lot No.	Test Start Date	5/31/90
Composition/Purity		Test End Date	6/4/90
Solvent	Coac.	Test Units (i.e., mg/L)	

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.0	7.0	7.0	7.0	
Control	8.0	8.1	8.1	8.1	
Highest Conc.	7.2	7.5	7.4	7.4	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed X 100 = %		
Total Number	0 : 50 X 100 = 0 %	0 : 50 X 100 = 0 %
Solvent Control	20 : 40 X 100 = 50 %	2 : 40 X 100 = 5 %
Control Length 78.0 mm	Solvent Control Length 68.6 mm	
Minimum Concentration to Inhibit Growth (MCIG)	0.015	MAS = 0.015

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	Failed variance	Failed variance	
LOEL	MAS	No MAS	
LC ₅₀	0.52	1.01	EC ₅₀ 0.29 / 0.52
95% Confidence limits	0.29 - 0.75 / 0.75 - 1.24		95% Confidence Limits 0.29 - 0.75
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			

POSITIVE CONTROL: 5 AMINONICOTINAMIDE (6-AN) RESULTS

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

FORT
BORIC ACIO W W/OMAS#2
LDF

FETAX SUMMARY SHEET

Test Material <u>NB</u>		Test No. <u>2</u>
Source <u>ILS</u>		Investigator <u>Fort</u>
CAS No.	Lot No.	Lab <u>SBL</u>
Composition/Purity		Test Start Date <u>8/24/94</u>
Solvent		Test End Date <u>8/28/94</u>
Conc.		Test Units (i.e., mg/ml) <u>mg/ml</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.5	7.5	7.4	7.4	
Control	7.8	7.8	7.9	7.8	
Highest Conc.	7.6	7.6	7.6	7.6	

FETAX CONTROL		MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed	X 100 = %		
Total Number		0 : 80 X 100 = 0 %	1 : 80 X 100 = 1.3 %
Solvent Control			
Control Length mm		Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		0.25	0.15

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	NA	0.075 / 0.075	Failed Williams
LOEL	NA	0.24 / 0.24	Failed Williams
LC ₅₀	0.42	0.40 - MAS	0.27
95% Confidence limits	0.40 - 0.45 0.37 - 0.43	95% Confidence Limits	0.26 - 0.28 0.24 - 0.27
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)		1.56	1.60

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 = %

FORT
BORIC ACID W WDMAS

FETAX SUMMARY SHEET

			TEST NO.	3
TEST MATERIAL	NB	INVESTIGATOR	FORT	
SOURCE	ITS	LAB	SBL	
CAS No.		LOT No.	TEST START DATE	8/31/94
COMPOSITION/PURITY		TEST END DATE		9/4/94
SOLVENT	CONC.	TEST UNITS (i.e., mg/mL)		mg/mL

pH	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock	7.6	7.6	7.7	7.7	
Control	7.9	7.9	7.9	7.9	
Highest Concentration	7.7	7.8	7.7	7.8	

FETAX CONTROL		MORTALITY	MALFORMATION
No. Dead or Malformed	X 100 = %	RECORD	RECORD
Total Number		0:80*100=0%	4:80*100=5%
Solvent Control			
Control Length mm		Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		0.25 / 0.24	128

TEST MATERIAL / COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	NA	0.15/0.15	WILLIAMS TEST
LOEL	NA	0.24/0.24	WILLIAMS TEST
LC50	0.55/0.44	EC50	0.24 / 0.20
95% Confidence limits	0.49-0.61	0.41-0.46	95% Confidence Limits 0.21-0.27 0.18-0.22
TEST TERATOGENIC INDEX (TI = LC50 / EC50)			2.29/2.20

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6 - AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg / L		
2500 mg / L		

ETHYLENE GLYCOL
DATA SUMMARY SHEETS
PHASE III-PART 3

Table 3. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCI/G and TI Values for Ethylene Glycol with Coefficients of Variation.

Laboratory	Without MAS*						With MAS					
	LC50			EC50			MCI/G			TI		
	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	Mean (mg/mL) and 95% CI	CV (%)	Mean and 95% CI
1	22.04	12.23	8.33	19.70	11.59	11.57	11.57	0.5	12.50	0.72	30.9	1.70
	19.21	13.22	12.57	16.85	11.7	11.57	11.57	0.5	12.50	0.72	30.9	1.46
	19.53	18.504 - 22.019	12.25	22.47	16.497 - 22.832	11.47	11.409 - 11.615	11.10	5.557 - 11.876	1.96	1.324 - 1.990	1.70
2	34.06	10.24	15.00	37.60	9.55	9.55	9.55	15.00	15.00	15.00	0.0	3.94
	27.35	10.78	10.47	27.68	12.4	9.90	8.69	14.5	15.00	15.00	0.0	4.01
	31.58	27.157 - 34.836	10.38	32.71	27.050 - 38.276	9.01	6.935 - 10.438	15.00	15.00	15.00	0.0	3.40
3	30.50	17.02	19.40	27.83	17.91	17.91	17.91	6.70	6.70	6.70	1.55	1.55
	29.43	17.95	16.61	17.51	18.7	19.40	17.23	12.2	2.00	9.37	78.5	0.90
	25.26	14.86	14.816 - 18.404	25.35	17.467 - 29.659	14.37	14.403 - 20.150	19.40	-0.819 - 19.552	1.76	0.896 - 1.911	1.76

*MAS=Metabolic Activation System

**CI=Confidence Interval

FINCH
 Ethylene Glycol w/o MAS #1

FETAX Summary Sheet

Test No. 208-0041

Test Material	NC w/o MAS			Investigator	DR. FINCH
Source	OSU			Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date:	16 MAY 94
Composition/Purity	08			Test End Date	20 MAY 94
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml)	100%

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7	7.5	6.8	7.1	
Control		7	7.1	7.2	7.1
Highest Concentration		7.1	7.4	7.1	7.2

No. Dead or Malformed X 100 = % Total Number	Mortality Record		Malformation Record	
	0 : 80	X 100 = 0%	2 : 80	X 100 = 2.5%
FETAX Control				
Solvent Control		X 100 =		X 100 =
Control Length (mm)	10.206		Solvent Control Length (mm)	J20
Minimum Concentration to Inhibit Growth (MCIG)	0.75			%

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	1.75	0.75	T-test
LOEL	N.A.	N.A.	T-test
LC50	1.986	EC50	1.102
95% CL	1.907	—	2.067
95% Confidence limits		0.909	*****
Test Teratogenic Index (TI = LC50/EC50):		1.80	
95% Confidence limits		1.48	— 2.19

Mendi,

this first NC has concentrations using 70
 The next two NC's are switched to mg/ml
 which is why the numbers don't appear
 to match.

Needs Comparison Check out

FINCH
Ethylene Glycol w MAS #1

FETAX Summary Sheet

Test No. 208-004A

Test Material	NC w/ MAS			Investigator	DR. FINCH
Source	OSU -			Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date:	16 MAY 94
Composition/Purity	C6			Test End Date	20 MAY 94
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml)	3.5%

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7	7.5	6.8	7.1	
Control		7	7.1	7.2	7.1
Highest Concentration		ND	7.2	7.2	7.2

No. Dead or Malformed				
X 100 = %				
Total Number	Mortality Record		Malformation Record	
FETAX Control	2 : 80	X 100 = 3%	0 : 78	X 100 = 0.0%
Solvent Control	:	X 100 =	:	X 100 =
Control Length (mm)	9.574	Solvent Control Length (mm)	J20	
Minimum Concentration to Inhibit Growth (MCIG)	0.5			%

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	1.5	0.1	T-test
LOEL	N.A.	N.A.	T-test
LC50	1.775	EC50	1.044
95% CL	1.141	--	2.762
		95% Confidence limits	0.802 ---- *****
Test Teratogenic Index (TI = LC50/EC50):		1.70	
95% Confidence limits		1.02	-- 2.85

C. FINCH
Ethylene Glycol w/ MAS #2

FETAX Summary Sheet

Test No. 208-005 I

Test Material	NC INACTIVATE		Investigator	DR. FINCH
Source	OSU		Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date: 20 MAR 95
Composition/Purity	C6		Test End Date	24 MAR 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml) MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	6.9	7.1	7	7	
Control		6.8	7	6.9	6.95
Highest Concentration		6.9	7.1	7.3	7.4

No. Dead or Malformed	
X 100 = %	
Total Number	
FETAX Control	
Solvent Control	

MALFORMATION EXCEED ASTM

Mortality Record		Malformation Record	
0 : 80	X 100 = 0%	8 : 80	X 100 = 10.0%
:	X 100 =	:	X 100 =
Control Length (mm)	9.941	Solvent Control Length (mm)	J20
Minimum Concentration to Inhibit Growth (MCIG)	12.5		MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	16.7	1.1	T-test
LOEL	N.A.	N.A.	T-test
LC50	19.207	EC50	13.218
95% CL	14.979 -- 24.629	95% Confidence limits	11.193 ---- *****
Test Teratogenic Index (TI = LC50/EC50):		1.45	
95% Confidence limits		1.08 -- 1.96	

FINCH
Ethylene Glycol w MAS #2

FETAX Summary Sheet

Test No. 208-005A

Test Material	NC ACTIVATED		Investigator	DR. FINCH
Source	OSU		Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date: 20 MAR 95
Composition/Purity	C6		Test End Date	24 MAR 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml) MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	6.9	7.1	7	7	
Control		6.8	7	6.9	6.95
Highest Concentration		6.9	6.975	6.9	

No. Dead or Malformed	X 100 = %		MALFORMATION EXCEED ASTM L	
Total Number	Mortality Record		Malformation Record	
FETAX Control	0 : 80	X 100 = 0%	10 : 80	X 100 = 12.5%
Solvent Control	:	X 100 =	:	X 100 =
Control Length (mm)	9.64	Solvent Control Length (mm) J20		
Minimum Concentration to Inhibit Growth (MCIG)	12.5		MG/M	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used	
NOEL	5.5	11.1	T-test	
LOEL	N.A.	N.A.	T-test	
LC50	16.852	EC50	11.569	
95% CL	15.379	--	18.467	95% Confidence limits 10.713 ---- *****
Test Teratogenic Index (TI = LC50/EC50):				1.46
95% Confidence limits				1.29 -- 1.64

plopdl19

FETAX Summary Sheet

Test No. 208-0061

Test Material	NC w/o MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Test Start Date:	01 MAY 95	Test End Date	05 MAY 95
Composition/Purity	C6	Test Units (i.e., mg/ml)	MG/ML
Solvent	B7	Conc.	E7

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.6	7.1	7.1	7.15	
Control		7.1	7.1	7.2	7.1
Highest Concentration		7.6	7.1	7.1	7.2

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length (mm) 9.876

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG) 11.1 MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	16.7	8.3	T-test
LOEL	N.A.	N.A.	T-test
LC50	19.533	EC50 12.251	
95% CL	16.112	-- 23.679	95% Confidence limits 11.413 ---- *****

Test Teratogenic Index (TI = LC50/EC50):

1.59 qq

95% Confidence limits

1.30

--

1.96

plopdl19

FETAX Summary Sheet

Test No. 208-006A

Test Material	NC ~~no~~ MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	01 MAY 95
Solvent	B7	Test End Date	05 MAY 95
Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.6	7.1	7.1	7.15	
Control		7.1	7.1	7.2	7.1
Highest Concentration		7	7	6.9	6.95

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length (mm) 9.523

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

11.1

MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	13.9	8.3	T-test
LOEL	N.A.	N.A.	T-test
LC50	22.468	EC50	11.468
95% CL	16.589	--	30.431
95% Confidence limits		10.893	----- *****
Test Teratogenic Index (TI = LC50/EC50):		1.96 qq	
95% Confidence limits		1.44	-- 2.66

BANTLE
Ethylene Glycol w/wc MAS #1

Chemical Code:	NC		
Compound:	Ethylene Glycol	Test No.:	10
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:	107-21-1	Laboratory:	OSU / Bantle
Lot No.:	23H0252	Start Date:	3-Jan-95
Glass / Plastic	Plastic	End Date:	7-Jan-95
Microsome lot No.:	JRR101	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX Controls	2.5	8.9
FETAX AB Controls		
MAS Controls	0	12.5

Cyclophosphamide

Positive Control	100		—
Negative Control	0		30

Without the Metabolic Activation System

LC50	34.06	EC50	10.24
95% CI	30.25 - 38.36	95% CI	5.92 - 17.72
MCIG = 15		TI	3.33
		95% CI	

With the Metabolic Activation System

LC50	37.60	EC50	9.55
95% CI	29.35 - 48.16	95% CI	6.34 - 14.40
MCIG = 15		TI	3.94
		95% CI	

CI = Confidence Interval

Chemical Code:	NC		
Compound:	Ethylene Glycol	Test No.:	11
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:	107-21-1	Laboratory:	OSU / Bantle
Lot No.:	23H0252	Start Date:	3-Jan-95
Glass / Plastic	Plastic	End Date:	7-Jan-95
Microsome lot No.:	M10	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX Controls	0	14.5
FETAX AB Controls	1.25	8.9
MAS Controls	0	12.5

Cyclophosphamide

Positive Control	100		--	
Negative Control	0		25	

Without the Metabolic Activation System

LC50	27.35	EC50	10.78
95% CI	25.60 - 29.23	95% CI	7.41 - 15.67
MCIG = 15		TI	2.54
		95% CI	

With the Metabolic Activation System

LC50	27.68	EC50	6.90
95% CI	26.19 - 29.24	95% CI	5.67 - 8.40
MCIG = 15		TI	4.01
		95% CI	

CI = Confidence Interval

FETAX Summary Sheet *Bantle*
Ethylene Glycol w/o MAS #3

Chemical Code:	NC		
Compound:	Ethylene Glycol	Test No.:	12
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:	107-21-1	Laboratory:	OSU / Bantle
Lot No.:	23H0252	Start Date:	5-Jan-95
Glass / Plastic	Plastic	End Date:	9-Jan-95
Microsome lot No.:	JRR101	Test Units:	mg/mL

	% Mortality ~~MAS~~		% Malformation ~~MAS~~	
FETAX Controls	7.5	~~---~~	8	~~---~~
FETAX AB Controls	7.5	~~---~~	1.2	~~---~~
MAS Controls	7.5	~~---~~	13.5	~~---~~

Cyclophosphamide

Positive Control	---	100	---	-
Negative Control	---	100	---	-

Without the Metabolic Activation System

LC50	31.58	EC50	10.38
95% CI	29.81 - 33.47	95% CI	4.59 - 23.46
$NCIG = 15$		TI	3.04
		95% CI	

With the Metabolic Activation System

LC50	32.71	EC50	9.61
95% CI	30.35 - 35.25	95% CI	4.27 - 21.45
$NCIG = 15$		TI	3.40
		95% CI	

CI = Confidence Interval

FETAX - U.S. ARMY ILS PROJECT (OKLA01 - RST901) RESULTS WITH NC

TEST	NO.	MAS	ENDPOINTS*		
			LC50	EC50	TI
Range**	R	N	-	-	-
Unactivated	1	N	18.54 (16.76-21.76)	20.42 (18.76-22.31)	0.91
Activated ***	FORT 1 1	Y	27.83/30.50 (25.85-29.96)/(28.20-33.20)	17.91/17.02 (15.68-20.45)	1.55/1.79
	FORT 2 2	Y	17.51/29.43 (15.04-20.39)/(26.48-32.70)	19.40/17.95 (NA)/(11.78-27.34)	0.90/1.63
	FORT 3 3	Y	25.35/25.26 (23.23-27.67)/(22.96-22.76)	14.37/14.86 (12.04-17.16)/(12.42-17.77)	1.76/1.70
					19.4/6.7
					2.0/> 19.4
					6.7/19.4
					16.67

* Expressed as mg/mL, except unactivated definitive test reported as %NM.
 ** Range test performed in accordance with the NTP protocol for the unactivated definitive test as documented accordingly.
 *** Activated / UNACTIVATED.

FETAX SUMMARY SHEET

FORT
Ethylene Glycol w/w MAS #1

Test No. 1

Test Material	NC	Investigator
Source		Lab
CAS No.	Lot No.	Test Start Date 5/31/94
Composition/Purity		Test End Date 6/4/94
Solvent	Conc.	Test Units (i.e., mg/dl)

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.5	7.7	7.9	7.7	
Control	8.0	8.1	8.2	8.2	
Highest Conc.	7.7	7.3	7.7	7.7	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %		
Solvent Control MAS	3 : 80 X 100 = 4 %	3 : 27 X 100 = 4 %
Control Length 791 mm MAS 7.5	0 : 40 X 100 = 0 %	2 : 40 X 100 = 5 %
Minimum Concentration to Inhibit Growth (MCIG)	19.4	w/MAS 6.7

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	19.4 / CON	6.7 / 6.7	TOXSTAT
LOEL	32.6 / 32.6	19.4 / 19.4	TOXSTAT
LC ₅₀	MAS = 27.83	EC ₅₀ MAS = 17.91	
95% Confidence Limits	25.85 - 33.20	14.75 - 19.65	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			
MAS 6.155 / 1.79			

POSITIVE CONTROL: 5 AMINONICOTINAMIDE (6-AN) RESULTS

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

FETAX SUMMARY SHEET

FORT
Ethylene Glycol w WOMAS F2

TEST NO. 2

TEST MATERIAL	NC	INVESTIGATOR	FORT
SOURCE	ILS	LAB	SBL
CAS No.	LOT No.	TEST START DATE	8/24/94
COMPOSITION/PURITY		TEST END DATE	8/28/94
SOLVENT	CONC.	TEST UNITS (i.e., mg/mL)	mg/mL

pH	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock	7.5	7.5	7.5	7.5	
Control	7.9	7.8	7.8	7.8	
Highest Concentration	7.7	7.7	7.8	7.7	

FETAX CONTROL	MORTALITY	MALFORMATION
No. Dead or Malform X 100 = %	RECORD	RECORD
Total Number	0:80*100=0%	0:80*100=0%
Solvent Control		
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	19.4	2.00

TEST MATERIAL / COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	NA	NA	FAILED ASSUMP
LOEL	NA	NA	FAILED ASSUMP
LC50	29.43/17.51	EC50	17.95/19.40
95% Confidence limits 26.48-32.70 15.04-20.39 95% Confidence Limits 11.78-27.34 NA			
TEST TERATOGENIC INDEX (TI = LC50 / EC50)		1.63/0.90	

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6 - AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg / L		
2500 mg / L		

FORT
Ethylene Glycol w/o MAS # 3

FETAX SUMMARY SHEET

Test No. 3

Test Material. NC	Investigator Fort
Source ILS	Lab SBL
CAS No.	Lot No.
Composition/Purity	Test Start Date 8/31/94
Solvent	Conc.
	Test End Date 9/4/94
	Test Units (i.e., mg/ml) mg/mL

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.7	7.7	7.7	7.8	
Control	7.9	7.8	7.8	7.9	
Highest Conc.	7.8	7.7	7.7	7.8	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number	X 100 = %	
	0 : 80 X 100 = 0 %	3 : 80 X 100 = 3.8 %
Solvent Control	1 : 40 X 100 = 2.5 %	2 : 39 X 100 = 5.1 %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	NA	2.0/2.0	Williams Test
LOEL	NA	6.7/6.7	NO MAS
LC ₅₀	25.24 / 25.35 MAS	EC ₅₀ 14.86 / 14.37	
95% Confidence Limits	22.46 - 22.78 22.23 - 27.67	95% Confidence Limits 12.42 - 17.77 12.04 - 17.16	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀) 1.70 / 1.76			

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 = _____ %

GLYCEROL
DATA SUMMARY SHEETS
PHASE III-PART 3

FINCH (11-G-95)
Glyxol w/o MAS # 1

FETAX Summary Sheet

Test No. 211-002 I

Test Material	ND w/o MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	84	Lot No.	E5
Composition/Purity	08	Test Start Date:	06 MAR 95
Solvent	87	Conc.	E7
		Test End Date	10 MAR 95
		Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	6.9	7.1	7	6.9	
Control		7	7.15	7.3	7
Highest Concentration		6.9	7.3	7.5	7.1

No. Dead or Malformed	MALFORMATION EXCEED ASTM LIMITS			
X 100 = %				
Total Number	Mortality Record		Malformation Record	
FETAX Control	Q : 80	X 100 = 0%	Z : 80	X 100 = 8.8%
Solvent Control	:	X 100 =	:	X 100 =
Control Length (mm)	10.012	Solvent Control Length (mm)	J20	
Minimum Concentration to Inhibit Growth (MCIG)	15			MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	15	10	T-test
LOEL	N.A.	N.A.	T-test
LC50	17.031	EC50	11.097
95% CL	11.662	95% Confidence limits	8.986
Test Teratogenic Index (TI = LC50/EC50):			
95% Confidence limits		0.99	2.37

FINCH
Glycerol w/w/o MAS #1

FETAX Summary Sheet

Test No. 211-002 A

Test Material	ND w/ MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	06 MAR 95
Solvent	B7	Test End Date	10 MAR 95
Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	6.9	7.1	7	6.9	
Control		7	7.15	7.3	7
Highest Concentration		7.1	ND	7	7.4

No. Dead or Malformed		
X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	4 : 80 X 100 = 5%	4 : 76 X 100 = 5.3%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm)	9.548	Solvent Control Length (mm) J20
Minimum Concentration to Inhibit Growth (MCIG)	N/A	10 MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	10	10	T-test
LOEL	N.A.	N.A.	T-test
LC50	11.402	EC50	10.582
95% CL	9.820	95% Confidence limits	8.893
Test Teratogenic Index (TI = LC50/EC50):			
95% Confidence limits		0.86	1.36

FINCH
Glycerol w/o MAS F 2

FETAX Summary Sheet

Test No. 211-003I

Test Material	ND w/o MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Test Start Date:	06 MAR 95	Test End Date	10 MAR 95
Composition/Purity	08	Test Units (i.e., mg/ml)	MG/ML
Solvent	B7	Conc.	E7

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	6.9	7.1	7	6.9	
Control		7	7.15	7.3	7
Highest Concentration		6.9	7.3	7.5	7.1

No. Dead or Malformed		
X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	2 : 80 X 100 = 3%	1 : 78 X 100 = 1.3%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm)	9.793	Solvent Control Length (mm) J20
Minimum Concentration to Inhibit Growth (MCIG)	15	MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	10	10	T-test
LOEL	N.A.	N.A.	T-test
LC50	10.320	EC50 10.213	
95% CL	8.056 — *****	95% Confidence limits 8.461 — *****	
Test Teratogenic Index (TI = LC50/EC50):			1.01
95% Confidence limits			0.74 — 1.38

FINCH
Glycerol w/MAS #2

FETAX Summary Sheet

Test No. 211-003A

Test Material	ND w/ MAS		Investigator	DR. FINCH
Source	OSU		Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date: 06 MAR 95
Composition/Purity	C6		Test End Date	10 MAR 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml) MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	6.9	7.1	7	6.9	
Control		7	7.15	7.3	7
Highest Concentration		7.1	7.3	7.3	7.4

No. Dead or Malformed					MALFORMATION EXCEED AST
X 100 = %					
Total Number	Mortality Record		Malformation Record		
FETAX Control	2 : 80	X 100 = 3%	12 : 78	X 100 = 15.4%	
Solvent Control	:	X 100 =	:	X 100 =	
Control Length (mm)	9.992	Solvent Control Length (mm)	J20		
Minimum Concentration to Inhibit Growth (MCIG)	10			MG/M	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	10	10	T-test
LOEL	N.A.	N.A.	T-test
LC50	6.241	EC50	11.255
95% CL	3.331	95% Confidence limits	7.387
Test Teratogenic Index (TI = LC50/EC50): 0.55			
95% Confidence limits 0.26 -- 1.18			

FINCH dated 11-6-95 #
glycerol w/omas #3

FETAX Summary Sheet

Test No. 211-004 INACTI

Test Material	ND w/omas	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No. B4	Lot No. E5	Test Start Date:	13 MAR 95
Composition/Purity	06	Test End Date	17 MAR 95
Solvent B7	Conc. E7	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	6.9	6.8	6.8	6.8	
Control		6.9	6.8	6.9	6.9
Highest Concentration		6.9	6.9	6.9	7

No. Dead or Malformed		
X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	0 : 80 X 100 = 0%	5 : 80 X 100 = 6.3%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm)	9.91	Solvent Control Length (mm) J20
Minimum Concentration to Inhibit Growth (MCIG)	8	MG/M

→ Now 10 see ND sheet

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	16	10	T-test
LOEL	N.A.	N.A.	T-test
LC50	17.156	EC50	11.351
95% CL	16.360 -- 17.991	95% Confidence limits	10.545 ---- *****
Test Teratogenic Index (TI = LC50/EC50):		1.51	
95% Confidence limits		1.38 --	1.65

*Finch
Glycol w MAS #3*

FETAX Summary Sheet

Test No. 211-004 A

Test Material	ND ACTIVATED		Investigator	DR. FINCH
Source	OSU		Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date: 13 MAR 95
Composition/Purity	C6		Test End Date	17 MAR 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml) MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	6.9	6.8	6.8	6.8	
Control		6.9	6.8	6.9	6.9
Highest Concentration		6.9	7.1	7	7.1

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM

Total Number

FETAX Control

Solvent Control

Mortality Record			Malformation Record		
4	: 80	X 100 = 5%	8	: 76	X 100 = 10.5%
:		X 100 =	:		X 100 =

Control Length (mm) 9.447

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG) N/A

6

MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used		
NOEL	12	10	T-test		
LOEL	N.A.	N.A.	T-test		
LC50	13.338	EC50	10.438		
95% CL	12.696	--	14.012	95% Confidence limits	6.346 ---- *****

Test Teratogenic Index (TI = LC50/EC50):

1.28

95% Confidence limits

0.78

--

2.11

ND

MCIG

211-002 I (w/o MAS)

15 $\mu\text{g/mL}$

211-002 A (w/MAS)

10 $\mu\text{g/mL}$

211-003 I (w/o MAS)

15 $\mu\text{g/mL}$

211-003 A (w/MAS)

10 $\mu\text{g/mL}$

211-004 I (w/o MAS)

8 $\mu\text{g/mL}$ - from data sheet

211-004 A (w/MAS)

6 $\mu\text{g/mL}$ JAB 7/15/96

Mendi,

Please check the back of
the Q pro data packages for
the data summary sheets.
If you still can't find them,
let me know and I'll send
them to you. Angela

FETAX Summary Sheet *BANTLE*
Glycerol w/ MAS #1

Chemical Code:	ND	Test No.:	3
Compound:	Glycerol	Investigator:	Mendi A. Hull
Source:	Sigma	Laboratory:	OSU / Bantle
CAS No.:	56-81-5	Start Date:	10-Jan-95
Lot No.:	34H0307	End Date:	14-Jan-95
Glass / Plastic	Plastic	Test Units:	mg/mL
Microsome lot No.:	JRR 101		

	% Mortality	% Malformation
FETAX Controls	5	11.8
FETAX AB Controls	2.5	10.3
MAS Controls	2.5	7.6

Cyclophosphamide

Positive Control	100	—
Negative Control	2.5	25.6

Without Metabolic Activation

LC50	17.99	EC50	12.13
95% CI	17.47-18.52	95% CI	11.66-12.62
		TI	1.48
		95% CI	

length: 0.8611 cm
 MCIG: 11.25

With Metabolic Activation

LC50	18.62	EC50	11.49
95% CI	18.10-19.16	95% CI	11.09-11.91
		TI	1.62
		95% CI	

length: 0.6846 cm
 MCIG: 12.5

CI = Confidence Interval

Glycerol w/ two MAS # 2

Chemical Code:	ND		
Compound:	Glycerol	Test No.:	4
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:	56-81-5	Laboratory:	OSU / Bantle
Lot No.:	34H0307	Start Date:	21-Feb-95
Glass / Plastic	Plastic	End Date:	25-Feb-95
Microsome lot No.:	A12	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	6.25 ~~25~~ ~~12~~	6.7
FETAX Controls	10 ~~10~~ ~~10~~	9.3
MAS Controls	7.5	21.6

Cyclophosphamide

Positive Control	100	—
Negative Control	0	17.1

Results

Without the Metabolic Activation System

LC50	20.68	EC50	10.96
95% CI	19.15 - 22.32	95% CI	9.78 - 12.28
Control length	1.006 cm	TI	1.89
MCIG	11.25 mg/mL	95% CI	

With the Metabolic Activation System

LC50	20.84	EC50	11.37
95% CI	18.74 - 23.18	95% CI	10.25 - 12.62
Control length	0.9504 cm	TI	1.83
MCIG	10.0 mg/mL	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

BANTLE
Glycerol w/wo MAS # 3

Chemical Code:	ND	Test No.:	5
Compound:	Glycerol	Investigator:	Mendi A. Hull
Source:	Sigma	Laboratory:	OSU / Bantle
CAS No.:	56-81-5	Start Date:	23-Feb-95
Lot No.:	34H0307	End Date:	27-Feb-95
Glass / Plastic	Plastic	Test Units:	mg/mL
Microsome lot No.:	~~A2~~ All		

	% Mortality	% Malformation
FETAX AB Controls	2.5	7.7
FETAX Controls	6.3	10.5
MAS Controls	0	14.6

Cyclophosphamide

Positive Control	100	—
Negative Control	0	12.5

Results

Without the Metabolic Activation System

LC50	18.64	EC50	11.35
95% CI	17.77 - 19.55	95% CI	9.96 - 12.95
Control length	1.003 cm	TI	1.64
MCIG	10 mg/mL	95% CI	

With the Metabolic Activation System

LC50	17.09	EC50	10.92
95% CI	15.42 - 18.94	95% CI	10.56 - 11.29
Control length	0.9409 cm	TI	1.57
MCIG	10 mg/mL	95% CI	

FETAX AB = FETAX antibiotic solution
 MAS = Metabolic Activation System
 CI = Confidence Interval

FETAX - U.S. ARMY ILS PROJECT (OKLA01-RSTS01) RESULTS WITH ND

TEST	NO.	MAS	ENDPOINTS*				MCIG
			LC50	EC50	TI		
Range**	R	N	5.5	> 1.0	< 5.5		> 1.0
			(NA)	(NA)			
Unactivated	1	N	1.06	0.90	1.18		1.1
			(1.04-1.08)	(0.87-0.93)			
Activated***	1	Y	1.18/0.80	0.55/0.60	2.15/1.33		0.29/0.29
			(1.14-1.22)/(0.73-0.87)	(0.48-0.63)/(0.51-0.70)			
	2	Y	0.88-0.66	0.44/0.46	2.00/1.43		0.11/0.29
			(0.80-0.98)/(0.59-0.72)	(0.39-0.51)/(0.41-0.50)			
	3	Y	1.17/0.80	0.41/0.54	2.85/1.48		0.11/0.29
			(1.11-1.23)/(0.73-0.89)	(0.36-0.48)/(0.48-0.62)			

*Converted to mg/ml by
multiplying by 12.5*

12.5 12.5
8 1.18
10.0 1.00
12.5
12.5
13.850

* Expressed as mg/ml
** Estimated based on graphical method.
*** Activated/Unactivated

FORT
Glycerol w/ no MFS #1

FETAX SUMMARY SHEET

Test Material ND		Investigator FORT
Source ILS		Lab 8BL
CAS No.	Lot No.	Test Start Date 9/7/94
Composition/Purity		Test End Date 9/11/94
Solvent	Conc.	Test Units (i.e., mg/kg) % (V/V)

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.6	7.6	7.5	7.5	
Control	7.9	7.8	7.8	7.9	
Highest Conc.	7.8	7.7	7.8	7.8	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
$\times 100 = \%$		
Total Number	0 : 80 $\times 100 = 0\%$	0 : 80 $\times 100 = 0\%$
~~Solvent~~ Control	0 : 40 $\times 100 = 0\%$	3 : 40 $\times 100 = 7.5\%$
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	0.29	0.29

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	NA	NA	Failed Assumptions
LOEL	NA	NA	Failed Assumptions
LC ₅₀	0.80 / 1.18 + MFS	EC ₅₀	0.60 / 0.55
95% Confidence limits	0.73 - 0.87 1.14 - 1.22	95% Confidence Limits	0.51 - 0.70 0.48 - 0.63
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)		1.33 / 2.15	

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	_____ : _____ $\times 100 =$ _____ %	_____ : _____ $\times 100 =$ _____ %
2500 mg/L	_____ : _____ $\times 100 =$ _____ %	_____ : _____ $\times 100 =$ _____ %

FETAX SUMMARY SHEET

FORT
Glycerol w/o MAS #2
Test No. 2

Test Material	ND	Investigator	Fort
Source	TLS	Lab	SBL
CAS No.		Lot No.	
Composition/Purity		Test Start Date	9/7/94
Solvent		Test End Date	9/11/94
		Test Units (i.e., mg/ml)	9. (V/V)

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.5	7.4	7.5	7.5	
Control	7.9	7.9	7.9	7.9	
Highest Conc.	7.8	7.7	7.8	7.8	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %		
MAS	2 : 80 X 100 = 2.5%	0 : 78 X 100 = 0%
Solvent Control	0 : 40 X 100 = 0%	3 : 40 X 100 = 7.5%
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	0.29	0.11

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.9/0.34	NA	Williams Test
LOEL	1.06/0.9	NA	
LC ₅₀	0.66/0.88 - MAS	EC ₅₀	0.46/0.44
95% Confidence Limits	0.58 - 0.72 0.80 - 0.99	95% Confidence Limits	0.41 - 0.50 0.39 - 0.51
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀) / 1.43 / 2.00			

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 = _____ %

FORT
Glycerol w/ no MAS # 3

FETAX SUMMARY SHEET

Test No. 3

Test Material <u>ND</u>		Investigator <u>Fort</u>
Source <u>ILS</u>		Lab <u>SBL</u>
CAS No.	Lot No.	Test Start Date <u>9/7/94</u>
Composition/Purity		Test End Date <u>9/11/94</u>
Solvent	Conc.	Test Units (i.e., mg/L) <u>90 (U/V)</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.4	7.5	7.4	7.4	
Control	7.9	7.8	7.9	7.9	
Highest Conc.	7.6	7.7	7.7	7.7	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %		
<u>MAS</u>	<u>3</u> : <u>80</u> X 100 = <u> </u> %	<u>2</u> : <u>77</u> X 100 = <u> </u> %
Solvent Control	<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 mm	Solvent Control Length mm <u>2/12/96</u>	
Minimum Concentration to Inhibit Growth (MCIG)	<u>0.29</u>	<u>0.11</u>

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.34 / 1.06	0.29 / 0.29	<u>Williams Test</u>
LOEL	0.90 / 1.51	0.34 / 0.34	
LC ₅₀	<u>0.80</u> / <u>1.17</u>	EC ₅₀ <u>0.54</u> / <u>0.41</u>	
95% Confidence limits	<u>0.73 - 0.89</u> <u>1.11 - 1.23</u>	95% Confidence Limits <u>0.48 - 0.62</u> <u>0.36 - 0.48</u>	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			<u>1.48</u> / <u>2.85</u>

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 = ___ %

SODIUM IODOACETATE
DATA SUMMARY SHEETS
PHASE III-PART 3

Table 5. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCIG and TI Values for Sodium Iodoacetate with Coefficients of Variation.

Laboratory	Without MAS*										With MAS									
	LC50		EC50		MCIG		TI		EC50		MCIG		TI		EC50		MCIG		TI	
	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean	CV (%)
	and 95% CI**		and 95% CI		and 95% CI		and 95% CI		and 95% CI		and 95% CI		and 95% CI		and 95% CI		and 95% CI		and 95% CI	
1	0.024	0.152	0.01	0.01	0.16	0.007	0.007	0.007	0.048	96.8	0.252	0.011	0.007	0.007	0.048	96.8	0.252	0.011	0.007	0.007
	0.034	0.167	0.0300	0.397	0.010	0.024	0.024	0.024	0.048	96.8	0.252	0.011	0.024	0.024	0.048	96.8	0.252	0.011	0.024	0.024
	0.442	0.103 - 0.437	0.740	0.051 - 0.743	NA	0.60	0.02 - 0.60	0.60	0.016 - 0.112	2.392	0.598 - 2.368	NA	0.113	0.016 - 0.112	2.392	0.598 - 2.368	NA	0.003	0.016 - 0.112	2.392
2	0.62	1.04	NA	NA	0.60	0.10	0.10	0.10	0.057	54.6	0.13	0.26	0.04	0.057	54.6	0.13	0.26	0.04	0.057	54.6
	0.16	0.327	63.7	0.655	NA	0.04	0.04	0.04	0.057	54.6	0.13	0.197	0.04	0.057	54.6	0.13	0.197	0.04	0.057	54.6
	0.20	0.038 - 0.615	0.27	0.039 - 1.271	NA	0.74	0.58 - 0.78	0.74	0.014 - 0.100	0.20	0.123 - 0.270	NA	0.03	0.014 - 0.100	0.20	0.123 - 0.270	NA	0.03	0.014 - 0.100	0.20
3	0.63	0.20	0.06	0.06	3.15	0.56	0.56	0.56	0.443	35.6	0.19	0.19	0.56	0.443	35.6	0.19	0.19	0.56	0.443	35.6
	0.60	0.663	10.5	0.267	0.064	0.06	0.06	0.06	0.443	35.6	0.19	0.180	0.06	0.443	35.6	0.19	0.180	0.06	0.443	35.6
	0.76	0.567 - 0.760	0.32	0.198 - 0.334	0.06	0.06	0.06	0.06	0.224 - 0.662	0.16	0.160 - 0.200	0.21	0.22	0.224 - 0.662	0.16	0.160 - 0.200	0.21	0.22	0.224 - 0.662	0.16
					2.38	1.960 - 3.154	2.38	1.960 - 3.154												

*MAS=Metabolic Activation System

**CI=Confidence Interval

FINCH
NaI w/o MAS # 1

FETAX Summary Sheet

Test No. 214-001 I

Test Material	NE W/O MAS		Investigator	DR. FINCH
Source	B5		Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date: 03 APR 95
Composition/Purity	C6			Test End Date 07 APR 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml) MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7	7.05	7.05	7	
Control		6.85	6.9	6.9	6.9
Highest Concentration		7.1	7.15	7.2	7.15

No. Dead or Malformed
X 100 = %

MALFORMATION EXCEED ASTM LIM

Total Number	Mortality Record		Malformation Record	
FETAX Control	3 : 80	X 100 = 4%	9 : 77	X 100 = 11.7%
Solvent Control	:	X 100 =	:	X 100 =
Control Length (mm)	9.932	Solvent Control Length (mm) J20		
Minimum Concentration to Inhibit Growth (MCIG)	0.01			MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used	
NOEL	N.A.	0.2	T-test	
LOEL	N.A.	N.A.	T-test	
LC50	0.024	EC50	0.152	
95% CL	0.007 -- 0.081	95% Confidence limits	0.110 ---- 0.211	
Test Teratogenic Index (TI = LC50/EC50):		0.16		
95% Confidence limits		0.04 -- 0.56		

F. NCH
NAI W N/MAS #02

FETAX Summary Sheet

Test No. 214-001

Test Material	NE W/MAS		Investigator	DR. FINCH
Source	B5		Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date: 03 APR 93
Composition/Purity	C6			Test End Date 07 APR 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml) MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH					
Stock	7	7.05	7.05	7	
Control		6.85	6.9	6.9	6.9
Highest Concentration		6.9	6.8	6.8	K13

No. Dead or Malformed X 100 = % Total Number	MALFORMATION EXCEED ASTM LIM									
	Mortality Record			Malformation Record						
	FETAX Control			FETAX Control						
	Solvent Control			Solvent Control						
	Control Length (mm)			Solvent Control Length (mm)						
	4	:	80	X 100 =	5%	22	:	76	X 100 =	28.9%
		:		X 100 =			:		X 100 =	
	9.041			120						
	Minimum Concentration to Inhibit Growth (MCIG)						N/A			
							MG/M			

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	N.A.	0.01	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.007	EC50	0.011
95% CL	0.000 -- 0.119	95% Confidence limits	0.001 ---- 0.100
Test Teratogenic Index (TI = LC50/EC50):		0.67	
95% Confidence limits		0.02 --	22.97

FINCH

NaI w/o MAS #2

plopdl19

FETAX Summary Sheet

Test No. 214-0021

Test Material	NE w/o MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Test Start Date:	24 APRIL 95		
Composition/Purity	C6	Test End Date	28 APRIL 95
Solvent	B7	Conc.	E7
Test Units (i.e., mg/ml)	MG/ML		

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7	7	7	7.05	
Control		6.9	6.9	6.9	6.9
Highest Concentration		7.2	7.1	7.2	7.3

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Mortality Record

Malformation Record

2 : 80

X 100 =

3%

5 : 78

X 100 =

6.4%

X 100 =

X 100 =

Control Length (mm) 10.09

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG) NONE

MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.05	0.8	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.034	EC50	0.300
95% CL	0.013	--	0.089
95% Confidence limits	0.009	----	9.887
Test Teratogenic Index (TI = LC50/EC50):			0.11 qq
95% Confidence limits	0.00	--	4.27

FINCH
NaIwMAS # 3

plopdl9

FETAX Summary Sheet

Test No. 214-002A

Test Material	NE w/ MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	24 APRIL 95
Solvent	B7	Test End Date	28 APRIL 95
Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7	7	7	7.05	
Control		6.9	6.9	6.9	6.9
Highest Concentration		7	7	7.2	6.95

No. Dead or Malformed	MORTALITY EXCEEDS ASTM LIMITS		MALFORMATION EXCEEDS ASTM LI	
X 100 = %				
Total Number	Mortality Record		Malformation Record	
FETAX Control	28 : 80	X 100 = 35%	10 : 52	X 100 = ***** 1970
Solvent Control		X 100 =		X 100 =
Control Length (mm)	9.114	Solvent Control Length (mm) J20		
Minimum Concentration to Inhibit Growth (MCIG)	NONE			MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.05	0.05	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.024	EC50	0.252
95% CL	0.002	--	0.346
95% Confidence limits		0.000	----- *****
Test Teratogenic Index (TI = LC50/EC50):		0.10 qq	
95% Confidence limits		0.00	-- *****

plopdl9

FETAX Summary Sheet

Test No. 214-0031

Test Material	NE w/o MAS		Investigator	DR. FINCH
Source	OSU		Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date: 24 APRIL 95
Composition/Purity	C6		Test End Date	28 APRIL 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml) MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7	7	7	7.05	
Control		6.9	6.9	6.9	6.9
Highest Concentration		7.2	7.1	7.2	7.3

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Mortality Record

Malformation Record

0 : 80

X 100 = 0%

5 : 80

X 100 = 6.3%

X 100 =

X 100 =

Control Length (mm) 9.866

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG) NONE

MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.05	0.7	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.442	EC50	0.740
95% CL	0.246	-- 0.794	95% Confidence limits 0.657 --- 0.835
Test Teratogenic Index (TI = LC50/EC50):			0.60 qq
95% Confidence limits			0.33 -- 1.08

FINCH
NaI w MAS#3

plopdl19

FETAX Summary Sheet

Test No. 214-003A

Test Material	NE w/ MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Test Start Date:	24 APRIL 95	Test End Date	28 APRIL 95
Composition/Purity	C6	Test Units (i.e., mg/ml)	MG/ML
Solvent	B7	Conc.	E7

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7	7	7	7.05	
Control		6.9	6.9	6.9	6.9
Highest Concentration		7	7	7	7

No. Dead or Malformed		
X 100 = %		
Total Number		
FETAX Control	Mortality Record	Malformation Record
Solvent Control	0 : 80 X 100 = 0%	2 : 80 X 100 = 2.5%
Control Length (mm)	9.719	Solvent Control Length (mm) J20
Minimum Concentration to Inhibit Growth (MCIG)	NONE	MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.01	0.2	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.113	EC50	2.392
95% CL	0.075	95% Confidence limits	0.021 ----- *****
Test Teratogenic Index (TI = LC50/EC50):			0.05 qq
95% Confidence limits			0.00 -- 5.41

BANTLE
NaI w/w MAS # 1

Chemical Code:	NE		
Compound:	Sodium Iodoacetate	Test No.:	2
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:		Laboratory:	OSU / Bantle
Lot No.:		Start Date:	6-Mar-95
Glass / Plastic	Plastic	End Date:	10-Mar-95
Microsome lot No.:	A11	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	0	3.75
FETAX Controls	0	1.23
MAS Controls	0	5.0

Cyclophosphamide

Positive Control	100	—
Negative Control	5	*

* see next sheet

Results

Without the Metabolic Activation System

LC50	0.62	EC50	1.04
95% CI	0.17 - 2.21	95% CI	0.32 - 3.38
Control length		TI	0.596
MCIG		95% CI	

With the Metabolic Activation System

LC50	0.10	EC50	0.26
95% CI	0.04 - 0.28	95% CI	0.05 - 1.21
Control length		TI	0.385
MCIG		95% CI	

FETAX AB = FETAX antibiotic solution
 MAS = Metabolic Activation System
 CI = Confidence Interval

BANTLE
NaI w/wo MAS #2

Chemical Code:	NE		
Compound:	Sodium Iodoacetate	Test No.:	3
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:		Laboratory:	OSU / Bantle
Lot No.:		Start Date:	14-Mar-95
Glass / Plastic	Plastic	End Date:	18-Mar-95
Microsome lot No.:	~~13~~ A13	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	7.5	8.1
FETAX Controls	15	1.5
MAS Controls	10	16.7

Cyclophosphamide

Positive Control	100	—
Negative Control	7.5	10.8

Results

Without the Metabolic Activation System

LC50	0.16	EC50	NC
95% CI	0.11 - 0.24	95% CI	
Control length		TI	
MCIG		95% CI	

NC = not calculatable.

With the Metabolic Activation System

LC50	0.04	EC50	0.13
95% CI	0.03 - 0.05	95% CI	0.05 - 0.35
Control length		TI	0.31
MCIG		95% CI	

FETAX AB = FETAX antibiotic solution
 MAS = Metabolic Activation System
 CI = Confidence Interval

Chemical Code:	NE	Test No.:	4
Compound:	Sodium Iodoacetate	Investigator:	Mendi A. Hull
Source:	Sigma	Laboratory:	OSU / Bantle
CAS No.:		Start Date:	27-Mar-95
Lot No.:		End Date:	31-Mar-95
Glass / Plastic	Plastic	Test Units:	mg/mL
Microsome lot No.:	B4 [REDACTED]		

	% Mortality	% Malformation
FETAX AB Controls	~~5.0~~ 5.0	8.1
FETAX Controls	11.3	9.8
MAS Controls	5.0	7.9

Cyclophosphamide

Positive Control	100	—
Negative Control	4.5	13.5

Results

Without the Metabolic Activation System

LC50	0.20	EC50	0.27
95% CI	0.14 - 0.29	95% CI	0.05 - 1.42
Control length	0.90074	TI	0.74
MCIG	nc	95% CI	

With the Metabolic Activation System

LC50	0.03	EC50	0.20
95% CI	0.005 - 0.241	95% CI	0.06 - 0.64
Control length	0.84062	TI	0.15
MCIG	nc	95% CI	

FETAX AB = FETAX antibiotic solution
MAS = Metabolic Activation System
CI = Confidence Interval

Reviewed by: _____

FETAX - U.S. ARMY ILS PROJECT (OKLA01-RSTS01) RESULTS WITH NE

ENDPOINTS*						
TEST	NO.	MAS	LC50	EC50	TI	MCIG
Range	R	N	0.71 (0.56-0.89)	0.17 (0.14-0.20)	4.18	0.5
Unactivated Definitive	1	N	0.64 (0.59-0.69)	0.21 (0.19-0.22)	3.04	>0.4
Activated** FORT 1	1	Y	0.66/0.63 (0.50-0.62)/(0.67-0.70)	0.19/0.20 (0.16-0.21)/(0.18-0.22)	2.95/3.15	0.20/0.064
FORT 2	2	Y	0.55/0.60 (0.50-0.60) (0.54-0.67)	0.19/0.28 (0.16-0.21) (0.25-0.30)	2.90/2.14	0.21/0.064
FORT 3	3	Y	0.22/0.76 (0.19-0.25) (0.65-0.68)	0.16/0.32 (0.13-0.20) (0.29-0.37)	2.36/2.38 ~~1.38/2.38~~	0.2/0.064

1.38 2.00
1.38 2.00
1.38 2.00
1.38 2.00
1.38 2.00

* Expressed as mg/mL
** Activated/Unactivated

FORT
NaI w/wo MAS# 1st E

FETAX SUMMARY SHEET

Test Material <u>NE</u>		Investigator <u>Fort</u>
Source <u>TLS</u>	Lab <u>SBI</u>	
CAS No.	Lot No.	Test Start Date <u>10/4/94</u>
Composition/Purity		Test End Date <u>10/8/94</u>
Solvent	Conc.	Test Units (i.e., mg/ml) <u>mg/ml</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	<u>7.4</u>	<u>7.4</u>	<u>7.5</u>	<u>7.5</u>	
Control	<u>7.9</u>	<u>7.9</u>	<u>7.9</u>	<u>7.9</u>	
Highest Conc.	<u>7.6</u>	<u>7.7</u>	<u>7.7</u>	<u>7.7</u>	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed X 100 = %	<u>0</u> : <u>80</u> X 100 = <u>0</u> %	<u>3</u> : <u>80</u> X 100 = <u>3.75</u> %
Total Number		
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) <u>0.064/0.2</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>NA</u>	<u>0.064/0.064</u>	<u>Williams Test</u>
LOEL	<u>NA</u>	<u>0.07/0.07</u>	<u>Williams</u>
LC ₅₀	<u>0.63/0.56</u>	<u>EC₅₀ 0.20/0.19</u>	
95% Confidence limits	<u>0.51 - 0.70</u> <u>0.50 - 0.62</u>	95% Confidence Limits <u>0.18 - 0.22</u> <u>0.16 - 0.21</u>	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			<u>3.15/2.95</u>

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> %

FORT
NaIw w/o MAS #2 - DT

FETAX SUMMARY SHEET

Test No. 2-MAS

Test Material	NE	Investigator	Font
Source	ILS	Lab	SBL
CAS No.		Test Start Date	10/4/94
Composition/Purity		Test End Date	10/8/94
Solvent		Test Units (i.e., mg/ml)	mg/mL

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.5	7.6	7.6	7.5	
Control	7.8	7.9	7.9	7.8	
Highest Conc.	7.8	7.8	7.7	7.7	

FETAX CONTROL		MORTALITY RECORD		MALFORMATION RECORD	
No. Dead or Malformed	X 100 = %	(2 : 80 X 100 = 2 %	1 : 80 X 100 = 1.3 %		
Total Number					
Solvent Control					
Control Length mm		Solvent Control Length mm			
Minimum Concentration to Inhibit Growth (MCIG)		0.064	0.21		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	—	0.07 / 0.067	Williams Test
LOEL	—	0.20 / 0.07	
LC ₅₀	0.60 / 0.55	EC ₅₀ 0.28 / 0.19	
95% Confidence limits	0.51 - 0.67 0.50 - 0.60	95% Confidence Limits 0.25 - 0.30 0.16 - 0.31	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			2.14 / 2.90

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 = — %

FORT
NaIw w/ MAS #3

FETAX SUMMARY SHEET

Test No. 3-MAS

Test Material	NE	Investigator	Fort
Source	TLS	Lab	SBL
CAS No.		Test Start Date	11/12/00
Composition/Purity		Test End Date	11/18/00
Solvent		Test Units (i.e., mg/ml)	mg/mL

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.4	7.5	7.5	7.5	
Control	7.9	7.9	7.9	7.9	
Highest Conc.	7.8	7.8	7.8	7.8	

FETAX CONTROL		MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed	X 100 = %		
Total Number		0 : 80 X 100 = 0 %	2 : 80 X 100 = 2.5 %
Solvent Control	MAS	0 : 40 X 100 = 0 %	0 : 40 X 100 = 0 %
Control Length	mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)		0.064	0.21

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	NA	0.06 / 0.02	Williams Test
LOEL	NA	0.2 / 0.06	
LC ₅₀	0.76	0.22	EC ₅₀ 0.32 / 0.16
95% Confidence limits	0.65 - 0.88	0.19 - 0.25	95% Confidence Limits 0.29 - 0.37
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			2.38 / 2.00

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 = _____ %

ACRYLAMIDE
DATA SUMMARY SHEETS
PHASE III-PART 3

Table 6. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCIG and TI Values for Acrylamide with Coefficients of Variation.

Laboratory	Without MAS*										With MAS									
	LC50					EC50					MCIG					TI				
	Mean (mg/mL) and 95% CI**	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI
1	0.144	0.066	0.066	0.070	0.070	2.19	2.50	2.51	10.4	0.081	0.102	15.3	0.017	0.042	0.03	0.032	33.8	0.03	0.037	25.7
	0.166	0.157	0.066	0.063	0.050	2.83	2.87	2.84	2.87	0.118	0.081	0.124	0.037	0.017	0.047	0.05	0.024	0.050	3.19	2.18 - 4.91
	0.160	0.144 - 0.170	0.056	0.056 - 0.069	0.005	0.004 - 0.079														
2	0.19	0.06	0.06	0.11	0.11	3.12				0.18	0.18	0.04	0.04	0.04	0.03	0.03	20.2	0.08	0.073	45.0
	0.18	0.269	0.05	0.057	0.08	3.60	4.08	4.08	40.2	0.18	0.240	35.4	0.06	0.047	0.047	0.037	12.9	0.015	0.015	0.0
	0.44	0.101 - 0.337	0.06	0.050 - 0.063	0.11	0.080 - 0.120	7.33	2.08 - 7.29		0.36	0.122 - 0.338	0.04	0.034	0.034 - 0.060	0.11	0.028	0.119	0.028	0.119	9.00
3	0.18	0.03	0.03	0.016	0.016	5.67				0.17	0.17	0.03	0.03	0.03	0.015	0.015	12.9	0.015	0.015	0.0
	0.25	0.223	0.05	0.040	0.015	3.1	5.00	5.56	7.5	0.19	0.167	12.3	0.04	0.037	0.037	0.037	12.9	0.015	0.015	0.0
	0.24	0.180 - 0.266	0.04	0.029 - 0.051	0.015	0.015 - 0.016	6.00	4.98 - 6.13		0.14	0.138 - 0.195	0.04	0.030	0.030 - 0.043	0.015	0.015	0.015	0.015	0.015	3.50

*MAS: Metabolic Activation System

**CI: Confidence Interval

plopdl19

FETAX Summary Sheet

Test No. 215-0011

Test Material	NF w/o MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Test Start Date:	27 MAR 95		
Composition/Purity	C6	Test End Date	31 MAR 95
Solvent	B7	Conc.	E7
Test Units (i.e., mg/ml)	MG/ML		

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.1	7.1	7.1	7.1	
Control		7.05	6.95	6.9	6.9
Highest Concentration		7.25	7.1	7.1	7.2

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Mortality Record

Malformation Record

0 : 80

X 100 =

0%

4 : 80

X 100 =

5.0%

X 100 =

X 100 =

Control Length (mm) 10.176

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

0.07

MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.11	0.05	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.144	EC50	0.066
95% CL	0.131	--	0.158
95% Confidence limits		0.027	0.162
Test Teratogenic Index (TI = LC50/EC50):		2.19	gg
95% Confidence limits		0.88	5.43

plopdl9

FETAX Summary Sheet

Test No. 215-001A

Test Material	NF w/ MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Test Start Date:	27 MAR 95		
Composition/Purity	C6	Test End Date	31 MAR 95
Solvent	B7	Conc.	E7
Test Units (i.e., mg/ml)	MG/ML		

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.1	7.1	7.1	7.1	
Control		7.05	6.95	6.9	6.9
Highest Concentration		7.2	6.8	6.8	6.8

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LI

Total Number

Mortality Record

Malformation Record

FETAX Control

0

80

X 100 =

0%

6

80

X 100 =

7.5%

Solvent Control

:

X 100 =

:

X 100 =

Control Length (mm)

9.67

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

0.03

MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.11	0.03	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.108	EC50	0.042
95% CL	0.073	--	0.158
95% Confidence limits	0.035	----	0.050

Test Teratogenic Index (TI = LC50/EC50):

2.56 qq

95% Confidence limits

1.68

--

3.91

plopdl19

FETAX Summary Sheet

Test No. 215-0021

Test Material	NF w/o MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	03 APRIL 95
Solvent	B7	Conc.	E7
		Test End Date	07 APRIL 95
		Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.1	7.15	7.1	7.1	
Control		7.3	6.9	6.9	6.9
Highest Concentration		7.3	7.2	7.2	7.2

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LI

Total Number

FETAX Control

Solvent Control

Control Length (mm) 10.544

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

0.05

MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.15	0.07	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.166	EC50	0.066
95% CL	0.143	--	0.192
		95% Confidence limits	0.055 --- 0.079

Test Teratogenic Index (TI = LC50/EC50):

2.50 qq

95% Confidence limits

1.98

--

3.16

plopdll9

FETAX Summary Sheet

Test No. 215-002A

Test Material	NF w/ MAS	Investigator	DR. FINCH
Source	qq	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Test Start Date:	03 APRIL 95	Test End Date	07 APRIL 95
Composition/Purity	C6	Test Units (i.e., mg/ml)	MG/ML
Solvent	B7	Conc.	E7

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.1	7.15	7.1	7.1	
Control		7.3	6.9	6.9	6.9
Highest Concentration		6.8	6.8	6.9	ND

No. Dead or Malformed

 $\times 100 = \%$

MALFORMATION EXCEED ASTM LI

Total Number

Mortality Record

Malformation Record

FETAX Control

6

80

 $\times 100 =$

8%

6

74

 $\times 100 =$

8.1%

Solvent Control

:

 $\times 100 =$

:

 $\times 100 =$

Control Length (mm)

9.739

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

0.03

MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.07	0.01	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.081	EC50	0.017
95% CL	0.065	--	0.102
95% Confidence limits		0.003	---- 0.102
Test Teratogenic Index (TI = LC50/EC50):		4.89	gg
95% Confidence limits		0.78	-- 30.50

plopdl9

FETAX Summary Sheet

Test No. 215-0031

Test Material	NF w/o MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	10 APRIL 95
Solvent	B7	Test End Date	14 APRIL 95
Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.1	7	7	7	
Control		6.9	6.9	6.8	6.8
Highest Concentration		7.2	7.2	7.15	7.2

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LI

Total Number

Mortality Record

Malformation Record

FETAX Control

0 : 80

X 100 = 0%

9 : 80

X 100 = *****

11:25%

Solvent Control

X 100 =

X 100 =

Control Length (mm) 10.075

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

0.005

MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.15	0.03	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.160	EC50	0.056
95% CL	0.155	--	0.164
		95% Confidence limits	0.045 ---- 0.071

Test Teratogenic Index (TI = LC50/EC50):

2.83

95% Confidence limits

2.26

--

3.54

plopdl19

F.N.C.
Reynolds W. 11/15/95

FETAX Summary Sheet

Test No. 215-003A

Test Material	NF w/ MAS	Investigator	DR. FINCH
Source	qq	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	10 APRIL 95
Solvent	B7	Test End Date	14 APRIL 95
Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.1	7	7	7	
Control		6.9	6.9	6.8	6.8
Highest Concentration		ND	6.9	6.9	6.9

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length (mm) 9.071

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

0.05

MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.09	0.01	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.118	EC50	0.037
95% CL	0.108	--	0.130
		95% Confidence limits	0.014 ---- 0.099
Test Teratogenic Index (TI = LC50/EC50):		3.19 qq	
95% Confidence limits		1.18	-- 8.59

FETAX Summary Sheet

Bantle
Acrylamide w w/o MAS #1

Chemical Code:	NF	Test No.:	1
Compound:	Acrylamide	Investigator:	Mendi A. Hull
Source:	Sigma	Laboratory:	OSU / Bantle
CAS No.:		Start Date:	14-Mar-95
Lot No.:		End Date:	18-Mar-95
Glass / Plastic	Plastic	Test Units:	mg/mL
Microsome lot No.:	A104		

	% Mortality	% Malformation
FETAX AB Controls	11.3	9.9
FETAX Controls	12.5	3.8
MAS Controls	5.0	13.2

Cyclophosphamide

Positive Control	100	—
Negative Control	15	5.9

Results

Without the Metabolic Activation System

LC50	0.187	EC50	0.06
95% CI		95% CI	0.05 - 0.07
Control length	0.86652 cm	TI	3.12
MCIG	0.11 mg/mL	95% CI	

With the Metabolic Activation System

LC50	0.184	EC50	0.04
95% CI		95% CI	0.037 - 0.049
Control length	0.85883 cm	TI	4.6
MCIG	0.03 mg/mL	95% CI	

FETAX AB = FETAX antibiotic solution
 MAS = Metabolic Activation System
 CI = Confidence Interval

FETAX Summary Sheet

Bantle
Acrylamide w w/o MAS

Chemical Code:	NF	Test No.:	2
Compound:	Acrylamide	Investigator:	Mendi A. Hull
Source:	Sigma	Laboratory:	OSU / Bantle
CAS No.:		Start Date:	27-Mar-95
Lot No.:		End Date:	31-Mar-95
Glass / Plastic	Plastic	Test Units:	mg/mL
Microsome lot No.:	B4		

	% Mortality	% Malformation
FETAX AB Controls	5.0	8.1
FETAX Controls	11.3	9.8
MAS Controls	5.0	7.9

Cyclophosphamide

Positive Control	100	—
Negative Control	7.5	13.5

Results

Without the Metabolic Activation System

LC50	0.18	EC50	0.05
95% CI		95% CI	0.05 - 0.06
Control length	0.86214 cm	TI	3.60
MCIG	0.08	95% CI	

With the Metabolic Activation System

LC50	0.176	EC50	0.06
95% CI		95% CI	0.05 - 0.06
Control length	0.84062	TI	2.93
MCIG	0.08	95% CI	

FETAX AB = FETAX antibiotic solution
 MAS = Metabolic Activation System
 CI = Confidence Interval

FETAX Summary Sheet

Bantle
Acrylamide w/o MAS

Chemical Code:	NF		
Compound:	Acrylamide	Test No.:	3
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:		Laboratory:	OSU / Bantle
Lot No.:		Start Date:	27-Mar-95
Glass / Plastic	Plastic	End Date:	31-Mar-95
Microsome lot No.:	J4	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	1.7	8.5
FETAX Controls	27.5	8.6
MAS Controls	5.0	7.9

Cyclophosphamide

Positive Control	100	—
Negative Control	10	16.7

Results

Without the Metabolic Activation System

LC50	0.44	EC50	0.06
95% CI		95% CI	0.05 - 0.08
Control length	0.87140	TI	7.33
MCIG	0.11	95% CI	

With the Metabolic Activation System

LC50	0.36	EC50	0.04
95% CI		95% CI	0.03 - 0.05
Control length	0.68457	TI	9.0
MCIG	0.11	95% CI	

FETAX AB = FETAX antibiotic solution
 MAS = Metabolic Activation System
 CI = Confidence Interval

FETAX - U.S. ARMY ILS PROJECT (OKLA01 - RSTS01) RESULTS WITH NF

ENDPOINTS*						
TEST	NO.	MAS	LC50	EC50	TI	MCIG
Range	R	N	0.11 (0.08-0.15)	0.05 (0.04-0.06)	220	0.005
Unactivated	1	N	0.15 (0.14-0.16)	0.05 (0.045-0.055)	3.00	0.035
Activated**	1	Y	0.17/0.18 (0.16-0.18)/0.18-0.19)	0.03/0.03 (0.03-0.04)/0.03-0.04)	5.67/6.00 6.00/5.67	0.015/0.016
	2	Y	0.19/0.25 (0.18-0.20)/(0.24-0.26)	0.04/0.05 (0.03-0.05)/(0.04-0.05)	4.75/5.00	0.015/0.015
	3	Y	0.14/0.24 (0.13-0.15)/(0.22-0.25)	0.04/0.04 (0.03-0.04)/(0.03-0.04)	3.50/6.00	0.015/0.015

* Expressed as mg/mL.
** Activated/Unactivated

FOR
Acrylamide w w's MAF=1

FETAX SUMMARY SHEET

Test Material NF		Investigator Fort	
Source ILS		Lab SBL	
CAS No.	Lot No.	Test Start Date 11/14/94	
Composition/Purity		Test End Date 11/18/94	
Solvent		Test Units (i.e., mg/ml) mg/ml	

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.6	7.6	7.7	7.7	
Control	7.9	7.9	7.9	7.9	
Highest Conc.	7.8	7.8	7.8	7.8	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number		
	0 : 80 X 100 = 0 %	4 : 80 X 100 = 0 %
Solvent Control MAS	0 : 40 X 100 = %	3 : 40 X 100 = 7.5 %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	0.016 0.015	

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	NAM	0.015 / 0.015	Williams Test
LOEL	NIA	0.016 / 0.016	"
LC ₅₀	0.18 / 0.17	EC ₅₀ 0.03 / 0.03	
95% Confidence limits 0.18 - 0.19 0.16 - 0.18		95% Confidence Limits 0.03 - 0.04 0.03 - 0.04	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			6.0 / 5.67

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 SUMMARY SHEET

1
FORT
Acrylonitrile w/w MAS # 2

Test Material <u>NF</u>		Investigator <u>Fort</u>
Source <u>ILS</u>		Lab <u>SBL</u>
CAS No.	Lot No.	Test Start Date <u>1/30/94</u>
Composition/Purity		Test End Date <u>12/2/94</u>
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	7.7	7.7	7.8	7.7	
Control	7.9	7.9	7.9	7.9	
Highest Conc.	7.8	7.8	7.8	7.9	

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>MAS</u>	<u>0</u> : <u>40</u> X 100 = <u>0</u> %	<u>0</u> : <u>40</u> X 100 = <u>0</u> %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>0.015</u> / <u>0.005</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	<u>NA</u>	<u>NA</u>	<u>Williams Test</u>
LOEL	<u>NA</u>	<u>NA</u>	
LC ₅₀	<u>0.25</u> / <u>0.19</u>	EC ₅₀ <u>0.05</u> / <u>0.04</u>	
95% Confidence limits	<u>0.24 - 0.26</u> <u>0.18 - 0.20</u>	95% Confidence Limits <u>0.04 - 0.05</u> <u>0.03 - 0.05</u>	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			<u>5.00</u> / <u>4.75</u>

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 = ____ %

FRT
Acrylamide w/ 6 MAS 3 RE

FETAX SUMMARY SHEET

Test No. 3-MAS

Test Material	NF	Investigator	Fort
Source	ILJ	Lab	SBL
CAS No.		Test Start Date	12/12/94
Lot No.		Test End Date	12/16/94
Composition/Purity		Test Units (i.e., mg/ml)	mg/mL
Solvent		Conc.	

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.6	7.6	7.8	7.7	
Control	7.9	7.9	7.8	7.9	
Highest Conc.	7.8	7.8	7.7	7.7	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %	2 : 80 X 100 = 2.5%	6 : 78 X 100 = 7.7%
Solvent Control	0 : 40 X 100 = 0%	3 : 40 X 100 = 7.5%
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	0.015	0.015

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	0.08 / 0.05	0.005 / 0.015	William Test
LOEL	0.1 / 0.08	0.015 / 0.016	
LC ₅₀	0.24 / 0.14	EC ₅₀ 0.04 / 0.04	
95% Confidence limits	0.22 - 0.25 0.13 - 0.15	95% Confidence Limits 0.03 - 0.04 0.03 - 0.04	

TEST TERATOGENIC INDEX (TI = LC₅₀ / EC₅₀) | 6.00 / 3.50

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 = ___ %

TRIETHYLENE GLYCOL DIMETHYLETHER
DATA SUMMARY SHEETS
PHASE III-PART 3

Table 7. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCIIG and TI Values for Triethylene Glycol Dimethylether with Coefficients of Variation.

Laboratory	Without MAS*										With MAS									
	LC50					EC50					MCIIG					TI				
	Mean (mg/ml.) and 95% CI**	CV (%)	Mean (mg/ml.) and 95% CI	CV (%)	Mean (mg/ml.) and 95% CI	CV (%)	Mean (mg/ml.) and 95% CI	CV (%)	Mean (mg/ml.) and 95% CI	CV (%)	Mean (mg/ml.) and 95% CI	CV (%)	Mean (mg/ml.) and 95% CI	CV (%)	Mean (mg/ml.) and 95% CI	CV (%)	Mean and 95% CI	CV (%)	Mean and 95% CI	CV (%)
1	17.60	9.35	3.00	1.88	16.45	7.09	2.00	2.22	17.08	18.00	9.41	21.0	6.00	3.67	1.31 - 6.02	2.22	1.44 - 2.54	2.22	1.43	20.0
	28.69	24.86	20.7	7.27	8.61	11.0	6.00	35.4	3.95	2.97	28.6	28.6	3.08	1.79 - 4.15	3.08	1.79 - 4.15	3.08	1.79 - 4.15	3.08	1.79 - 4.15
	28.30	17.74 - 31.98	9.20	7.29 - 9.92	3.00	2.04 - 5.96	3.00	2.04 - 5.96	3.00	2.04 - 5.96	3.00	2.04 - 5.96	3.00	2.04 - 5.96	3.00	2.04 - 5.96	3.00	2.04 - 5.96	3.00	2.04 - 5.96
2	26.94	8.13	4.00	1.75 - 13.75	5.81	2.99 - 5.86	5.81	2.99 - 5.86	5.81	2.99 - 5.86	5.81	2.99 - 5.86	5.81	2.99 - 5.86	5.81	2.99 - 5.86	5.81	2.99 - 5.86	5.81	2.99 - 5.86
	28.41	29.86	10.5	6.88	6.97	13.2	11.50	7.75	48.4	4.13	4.42	23.4	15.23	14.56	39.5	3.10	4.27	38.3	4.00	3.25
	34.23	25.50 - 34.22	5.89	5.70 - 8.24	4.00	1.75 - 13.75	5.81	2.99 - 5.86	5.81	2.99 - 5.86	5.81	2.99 - 5.86	5.81	2.99 - 5.86	5.81	2.99 - 5.86	5.81	2.99 - 5.86	5.81	2.99 - 5.86
3	23.43	8.17	8.10	2.87	13.97	5.69	2.50	2.46	10.66	14.02	4.61	37.5	2.50	2.50	2.05 - 4.45	2.30	1.98 - 4.98	2.30	1.98 - 4.98	2.30
	26.76	26.93	10.9	5.15	6.41	20.0	8.10	8.10	0.0	5.20	4.42	24.8	10.66	14.02	19.7	2.17	4.61	37.5	2.50	2.50
	30.60	22.87 - 30.99	5.90	4.63 - 8.19	8.10	8.10 - 8.10	8.10	8.10 - 8.10	5.19	2.90 - 5.94	5.19	2.90 - 5.94	17.43	10.19 - 17.85	5.96	2.21 - 7.00	2.50	#NUM!	2.50	#NUM!

*MAS-Metabolic Activation System

**CI - Confidence Interval

FINCH
Triethylene Glycol w/o MAS # 1

plopdl19

FETAX Summary Sheet

Test No. **216-00419**

Test Material	NG w/o MAS!		Investigator	DR. FINCH	
Source	OSU		Laboratory	USABRD	
CAS No.	B4	Lot No.	E5	Test Start Date:	18 DEC 95
Composition/Purity	C6		Test End Date	22 DEC 95	
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	6.70	6.70	6.90	6.90	
Control		7.05	7.00	7.00	7.00
Highest Concentration		6.75	6.95	7.00	6.95

No. Dead or Malformed		X 100 = %	
Total Number	Mortality Record		Malformation Record
FETAX Control	0 : 80	X 100 = 0%	1 : 80 X 100 = 1.3%
Solvent Control	:	X 100 =	: X 100 =
Control Length (mm)	10.074	Solvent Control Length (mm)	J20
Minimum Concentration to Inhibit Growth (MCIG)	3 MG/ML		

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	15	3	T-test
LOEL	N.A.	N.A.	T-test
LC50	17.602	EC50	9.351
95% CL	16.378	--	18.918
95% Confidence limits		6.493	13.468
Test Teratogenic Index (TI = LC50/EC50):		1.88 qq	
95% Confidence limits		1.30	2.73

Replaces NG 216-001 Inactivated

99

FETAX Summary Sheet

Test No. 216-001A 1

Test Material	NG w/ MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	01 MAY 95
Solvent	B7	Conc.	E7
		Test End Date	05 MAY 95
		Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.1	7	7.1	7.05	
Control		7.1	7.05	7.15	7.05
Highest Concentration		plopdl9	7	6.95	6.9

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Mortality Record

Malformation Record

0 : 80

X 100 = 0%

2 : 80

X 100 = 2.5%

X 100 =

X 100 =

Control Length (mm) 9.347

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

2

MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	14	4	T-test
LOEL	N.A.	N.A.	T-test
LC50	16.447	EC50	7.088
95% CL	14.402	95% Confidence limits	6.394 --- 7.858
Test Teratogenic Index (TI = LC50/EC50):			2.32 qq
95% Confidence limits			1.96 --- 2.74

p10pd119

FETAX Summary Sheet

Test No. 216-0021

Test Material	NG w/o MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	15 MAY 95
Solvent	B7	Conc.	E7
		Test End Date	19 MAY 95
		Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.3	7	7.05	7	
Control		6.9	7	6.95	6.95
Highest Concentration		7.2	7.1	7	7.2

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Mortality Record

Malformation Record

0 : 80

X 100 = 0%

4 : 80

X 100 = 5.0%

X 100 =

X 100 =

Control Length (mm) 9.358

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

6

MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	21	6	T-test
LOEL	N.A.	N.A.	T-test
LC50	28.686	EC50	7.270
95% CL	21.683	--	37.951
		95% Confidence limits	6.196 --- 8.530
Test Teratogenic Index (TI = LC50/EC50):			3.95 qq
95% Confidence limits			2.86 -- 5.45

plopdl19

FETAX Summary Sheet

Test No. 216-002A

Test Material	NG w/ MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	15 MAY 95
Solvent	B7	Test End Date	19 MAY 95
Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.3	7	7.05	7	
Control		6.9	7	6.95	6.95
Highest Concentration		6.95	6.9	6.9	6.9

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Mortality Record

Malformation Record

0 : 80

X 100 = 0%

2 : 80

X 100 = 2.5%

X 100 =

X 100 =

Control Length (mm) 9.284

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

6

MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	9	N.A.	T-test
LOEL	N.A.	N.A.	T-test
LC50	17.081	EC50	11.909
95% CL	15.547	95% Confidence limits	0.250 ---- *****
Test Teratogenic Index (TI = LC50/EC50):			
95% Confidence limits			
0.03 -- 1.43 qq 68.49			

plopdll9

FETAX Summary Sheet

Test No. 216-0031

Test Material	NG w/o MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	19 JUNE 95
Solvent	B7	Test End Date	23 JUNE 95
Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.43	7.19	7.33	6.82	
Control		7.1	7.43	7.1	6.95
Highest Concentration		7.78	7.41	7.25	7.31

No. Dead or Malformed				
X 100 = %				
Total Number				
FETAX Control				
Solvent Control				
Control Length (mm)	9.892	Solvent Control Length (mm)	J20	
Minimum Concentration to Inhibit Growth (MCIG)	3		MG/M	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	27	9	T-test
LOEL	N.A.	N.A.	T-test
LC50	28.303	EC50	9.199
95% CL	25.962	95% Confidence limits	2.480 ----- *****
Test Teratogenic Index (TI = LC50/EC50):			
95% Confidence limits			
0.83 ----- 3.08 qq ----- 11.44			

FINCH
Triethylene Glycol w MAS #3

plopdl19

FETAX Summary Sheet

Test No. 216-003A

Test Material	NG w/ MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No. B4	Lot No. E5	Test Start Date:	19 JUNE 95
Composition/Purity C6		Test End Date	23 JUNE 95
Solvent B7	Conc. E7	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7.43	7.19	7.33	6.82	
Control		7.1	7.43	7.1	6.95
Highest Concentration		7.43	7.66	7.82	7.41

No. Dead or Malformed	MALFORMATION EXCEED ASTM LI			
X 100 = %				
Total Number	Mortality Record		Malformation Record	
FETAX Control	0 : 80	X 100 = 0%	6 : 80	X 100 = 7.5%
Solvent Control		X 100 =		X 100 =
Control Length (mm)	9.714	Solvent Control Length (mm) J20		
Minimum Concentration to Inhibit Growth (MCIG)	3			MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	21	9	T-test
LOEL	N.A.	N.A.	T-test
LC50	20.476	EC50	9.224
95% CL	19.308	95% Confidence limits	7.605 ---- *****
Test Teratogenic Index (TI = LC50/EC50):			
95% Confidence limits		1.81	2.22 qq

~~FETAX~~ BANTLE
Triethylene Glycol w/o MAS

Chemical Code:	NG		
Compound:	Triethylene Glycol Dim	Test No.:	1
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:	107-21-1	Laboratory:	OSU / Bantle
Lot No.:	23H0252	Start Date:	30-Mar-95
Glass / Plastic	Plastic	End Date:	3-Apr-95
Microsome lot No.:	M12	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	0	2.5
FETAX Controls	0	2.5
MAS Controls	0	2.5

Cyclophosphamide

Positive Control	100	—
Negative Control	0	12.2

Results

Without the Metabolic Activation System

LC50	26.94	EC50	8.13
95% CI	23.93 - 30.34	95% CI	7.65 - 8.64
Control length		TI	3.33
MCIG	?	95% CI	

With the Metabolic Activation System

LC50	21.25	EC50	6.58
95% CI	8.36 - 54.0	95% CI	6.19 - 6.97 7.0
Control length		TI	3.23
MCIG	?	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

Chemical Code:	NG	Test No.:	5
Compound:	1,2-Ethylene Glycol Dim	Investigator:	Mendi A. Hull
Source:	Sigma	Laboratory:	OSU / Bantle
CAS No.:	107-21-1	Start Date:	8-Jul-95
Lot No.:	23H0252	End Date:	12-Jul-95
Glass / Plastic	Plastic	Test Units:	mg/mL
Microsome lot No.:	B3		

	% Mortality	% Malformation
FETAX AB Controls	0	5
FETAX Controls	1.25	5
MAS Controls	0	12

Cyclophosphamide

Positive Control	100	—
Negative Control	0	33

Results

Without the Metabolic Activation System

LC50	28.41	EC50	6.88
95% CI	27.02 - 29.86	95% CI	5.58 - 8.48
Control length	0.86109	TI	4.13
MCIG	11.5	95% CI	

With the Metabolic Activation System

LC50	15.23	EC50	3.10
95% CI	6.02 - 38.56	95% CI	0.85 - 11.31
Control length	0.89757	TI	4.91
MCIG	4.0	95% CI	

FETAX AB = FETAX antibiotic solution
 MAS = Metabolic Activation System
 CI = Confidence Interval

Chemical Code:	NG	Test No.:	6
Compound:	<i>15</i> Diethylene Glycol Dim	Investigator:	Mendi A. Hull
Source:	Sigma	Laboratory:	OSU / Bantle
CAS No.:	107-21-1	Start Date:	12-Jul-95
Lot No.:	23H0252	End Date:	16-Jul-95
Glass / Plastic	Plastic	Test Units:	mg/mL
Microsome lot No.:	J5		

	% Mortality	% Malformation
FETAX AB Controls	2.5	6.4
FETAX Controls	1.3	2.5
MAS Controls	7.5	5.4

Cyclophosphamide

Positive Control	100	-
Negative Control	0	17.1

Results

Without the Metabolic Activation System

LC50	34.23	EC50	5.89
95% CI	32.82 - 35.69	95% CI	4.97 - 6.97
Control length	0.93543 cm	TI	5.81
MCIG	4.0	95% CI	

With the Metabolic Activation System

LC50	7.2*	EC50	3.13
95% CI		95% CI	2.82 - 3.47
Control length	0.90029 cm	TI	2.30
MCIG	2.5	95% CI	

*graphing method

FETAX AB = FETAX antibiotic solution
 MAS = Metabolic Activation System
 CI = Confidence Interval

FETAX - U.S. ARMY ILS PROJECT (OKLA01-RSTS01) RESULTS WITH NG

TEST	NO.	MAS	ENDPOINTS*			MCIG
			LC50	EC50	TI	
Range	R	N	14.65 (12.0-17.9)	6.24 (5.3-7.34)	2.35	2.5
Unactivated	I	N	25.21 (24.26-26.21)	11.03 (10.63-11.44)	2.29	5.0
Activated**	1	Y	13.74/31.24 (13.02-14.49)/(30.11-32.41)	5.46/10.11 (4.90-6.09)/(9.21-11.09)	3.09/2.52	2.5/3.5
FORT 1x		Y	13.97/23.43 (13.58-14.38)/(22.50-22.41)	5.69/8.17 (5.01-6.48)/(7.52-8.88)	2.46/2.87	2.5/8.1
		Y	10.68/26.76 (9.55-11.91)/(24.90-28.75)	2.17/5.15 (1.84-2.55)/(4.32-6.14)	4.91/5.20	2.5/8.1
		Y	17.43/30.60 (16.42-18.49)/(28.21-32.06)	5.96/5.90 (5.15-6.90)/(5.03-6.92)	2.92/5.19	2.5/8.1

* Expressed as mg/mL.
** Activated/Unactivated.

FOR
Triethylene Glycol w/w/m/s #1
✓ JF

FETAX SUMMARY SHEET

Test Material <u>NIG</u>		Investigator <u>Fort</u>
Source <u>ILS</u>		Lab <u>SBL</u>
CAS No.	Lot No.	Test Start Date <u>12/5/94</u>
Composition/Purity		Test End Date <u>12/9/94</u>
Solvent	Conc.	Test Units (i.e., mg/ml) <u>mg/mL</u>

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.7	7.7	7.7	7.6	
Control	7.8	7.8	7.9	7.9	
Highest Conc.	7.7	7.8	7.8	7.8	

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %		
	3 : 80 X 100 = 3.8 %	3 : 77 X 100 = 3.9 %
Solvent Control	2 : 40 X 100 = 5 %	3 : 38 X 100 = 7.9 %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	8.1	2.5

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	18.5 / 11.0	3.5 / 2.5	Williams test
LOEL	20.9 / 18.5	8.1 / 3.5	
LC ₅₀	23.43 / 13.97	EC ₅₀ 8.17 / 5.69	
95% Confidence Limits	22.58 - 24.41 13.58 - 14.38	95% Confidence Limits 7.52 - 8.88 5.01 - 6.48	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			2.87 / 2.46

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 = ___ %

FOR: Triethylene glycol W. W. MAS-3

FETAX SUMMARY SHEET

Test No. MAS-3

Test Material - NG	Investigator Fort
Source ILS	Lab SBL
CAS No.	Lot No.
Composition/Purity	Test Start Date 12/12/94
Solvent	Conc.
	Test End Date 12/16/94
	Test Units (i.e., mg/ml) mg/mL

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.7	7.7	7.5	7.6	
Control	7.8	7.8	7.9	7.9	
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed X 100 = %		
Total Number	0 : 80 X 100 = 0 %	7 : 80 X 100 = 8.8 %
Solvent Control	0 : 40 X 100 = 0 %	3 : 40 X 100 = 7.5 %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	8.1	2.5

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	NA	1.1 / 0.0	Williams Test
LOEL	NA	2.5 / 1.1	
LC ₅₀	26.76 / 10.66	EC ₅₀ 5.15 / 2.17	
95% Confidence limits	24.90 - 28.75 9.55 - 11.91	95% Confidence Limits 4.32 - 6.14 1.94 - 2.55	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			5.20 / 4.91

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 = ___ %

FGR: Triethylene glycol w/o MAS

FETAX SUMMARY SHEET

Test No. 4-MAS

Test Material	NG	Investigator	Fort
Source	ILS	Lab	SBL
CAS No.		Lot No.	
Composition/Purity		Test Start Date	12/20/94
Solvent		Test End Date	12/24/94
Conc.		Test Units (i.e., mg/mL)	ml/mL

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.6	7.6	7.6	7.6	
Control	7.8	7.8	7.8	7.8	
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %	0 : 80 X 100 = 0 %	2 : 80 X 100 = 2.5 %
Solvent Control MAS	2 : 40 X 100 = 5 %	3 : 38 X 100 = %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	8.1	2.5

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	NA	1.1 / 0.0	Williams Test
LOEL	NA	2.5 / 1.1	
LC ₅₀	30.60 / 17.43	EC ₅₀ 5.90 / 5.96	
95% Confidence Limits	29.21 - 32.06 16.42 - 18.49	95% Confidence Limits 5.03 - 6.92 5.15 - 6.70	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			5.19 / 2.92

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 = ____ %

DIETHYLENE GLYCOL
DATA SUMMARY SHEETS
PHASE III-PART 3

Table 8. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCIG and TI Values for Diethylene Glycol with Coefficients of Variation.

Laboratory	Without MAS*					With MAS				
	LC50		EC50		TI	LC50		EC50		MCIG
	Mean (mg/mL) and 95% CI**	CV (%)	Mean (mg/mL) and 95% CI	CV (%)		Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	
1	30.58	21.08	15.00	1.45	23.77	18.84	20.00	126	1.26	66.3
	28.90	30.03	9.3	20.00	21.51	23.81	42.7	13.50	68.1	4.76
	30.62	28.92 - 31.14	5.00	4.69 - 21.98	26.16	21.19 - 26.44	5.50	0.76 - 26.24	1.30	2.39
2	43.00	17.20	19.00	2.50	34.36	14.39	11.00	18.00	29.7	2.08
	45.36	39.88	2.5	24.00	36.01	29.68	9.1	18.00	29.7	2.08
	31.29	31.36 - 48.41	13.00	12.43 - 24.90	18.67	18.85 - 40.51	14.31	10.58 - 35.42	1.30	2.39
3	34.66	10.23	2.50	3.39	27.30	10.38	2.50	2.50	0.0	3.66
	30.72	32.48	5.10	36.4	21.8	24.34	5.3	8.65	17.7	2.50
	32.07	30.22 - 34.75	2.50	1.67 - 5.07	27.34	24.18 - 28.27	8.92	6.52 - 10.78	2.50	3.07

*MAS: Metabolic Activation System

**CI: Confidence Interval

plopdll9

FETAX Summary Sheet

Test No. 217-0011^s

Test Material	NH w/o MAS			Investigator	DR. FINCH
Source	OSU			Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date:	15 MAY 95
Composition/Purity	C6			Test End Date	19 MAY 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.1	7	7.1	7.1	
Control		6.9	7	6.95	6.9
Highest Concentration		7.2	7	7.2	7.1

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LIM

Total Number

Mortality Record

Malformation Record

FETAX Control

0 : 80

X 100 = 0%

8 : 80

X 100 = 10.0%

Solvent Control

:

X 100 =

:

X 100 =

Control Length (mm) 10.141

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

15

MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used		
NOEL	25	15	T-test		
LOEL	N.A.	N.A.	T-test		
LC50	30.578		EC50	21.083	
95% CL	27.685	-- 33.774	95% Confidence limits	8.981	--- 49.491
Test Teratogenic Index (TI = LC50/EC50):					
95% Confidence limits					
				1.45	
				0.61	-- 3.42

plopdl19

FETAX Summary Sheet

Test No. 217-001A

Test Material	NH w/ MAS		Investigator	DR. FINCH
Source	OSU		Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date: 15 MAY 95
Composition/Purity	C6		Test End Date	19 MAY 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml) MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.1	7	7.1	7.1	
Control		6.9	7	6.95	6.9
Highest Concentration		6.85	6.9	6.9	6.9

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LIM

Total Number

Mortality Record

Malformation Record

FETAX Control

2 : 80 X 100 = 3%

8 : 78 X 100 = 10.3%

Solvent Control

X 100 =

X 100 =

Control Length (mm) 9.917

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG) 20

MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	20	15	T-test
LOEL	N.A.	N.A.	T-test
LC50	23.770	EC50	18.841
95% CL	21.783	-- 25.937	95% Confidence limits 16.368 ---- 21.689
Test Teratogenic Index (TI = LC50/EC50):			1.26
95% Confidence limits			1.07 -- 1.49

p10pdl19

FETAX Summary Sheet

Test No. 217-0031

Test Material	- NH w/o MAS			Investigator	DR. FINCH
Source	OSU			Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date:	17 JULY 95
Composition/Purity	C6			Test End Date	21 JULY 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	8.02	8.36	7.4	7.43	
Control		7.2	8.24	7.42	7.37
Highest Concentration		8.16	8.51	7.45	7.52

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LIMIT

Total Number

FETAX Control

Solvent Control

Control Length (mm) 10.537

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

20

MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	25	15	T-test
LOEL	N.A.	N.A.	T-test
LC50	28.900	EC50	16.940
95% CL	26.692	--	31.290
95% Confidence limits		12.712	22.575

Test Teratogenic Index (TI = LC50/EC50):

1.71

95% Confidence limits

1.27

--

2.30

FINCH
Dibutylone Glycol w MAS = 2

qq

plopdl19

FETAX Summary Sheet

Test No. 217-003A

Test Material	NH w/ MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Test Start Date:	17 JULY 95	Test End Date	21 JULY 95
Composition/Purity	C6	Test Units (i.e., mg/ml)	MG/ML
Solvent	B7	Conc.	E7

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	8.02	8.36	7.4	7.43	
Control		7.2	8.24	7.42	7.37
Highest Concentration		8.2	7.32	7.43	7.16

No. Dead or Malformed		
X 100 = %		
Total Number	Mortality Record	Malformation Record
FETAX Control	0 : 80 X 100 = 0%	2 : 80 X 100 = 2.5%
Solvent Control	: X 100 =	: X 100 =
Control Length (mm)	9.162	Solvent Control Length (mm) J20
Minimum Concentration to Inhibit Growth (MCIG)	20	MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	20	10	T-test
LOEL	N.A.	N.A.	T-test
LC50	21.514	EC50	15.886
95% CL	19.238 -- 24.060	95% Confidence limits	15.886 --- 15.886
Test Teratogenic Index (TI = LC50/EC50):			1.35 qq
95% Confidence limits			1.21 -- 1.51

plopdl9

FETAX Summary Sheet

Test No. 217-0051*

Test Material	NH w/o MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Test Start Date:	18 SEPT 95	Test End Date	22 SEPT 95
Composition/Purity	C6	Test Units (i.e., mg/ml)	MG/ML
Solvent	B7	Conc.	E7

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.30	7.30	7.30	7.15	
Control		7.20	7.10	7.30	7.10
Highest Concentration		7.30	7.25	7.25	7.25

No. Dead or Malformed

X 100 = %

Total Number

Mortality Record

Malformation Record

FETAX Control

0 : 80

X 100 =

0%

2 : 80

X 100 =

2.5%

Solvent Control

:

X 100 =

:

X 100 =

Control Length (mm) 10.149

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

5 MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	25	15	T-test
LOEL	N.A.	N.A.	T-test
LC50	30.617	EC50	18.187
95% CL	29.432	—	31.850
95% Confidence limits	11.460	—	28.865

Test Teratogenic Index (TI = LC50/EC50):

1.68

95% Confidence limits

1.06

—

2.68

mz/king 28 Dec 95

plopdl19

FETAX Summary Sheet

Test No. 217-005A

Test Material	NH W/ MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	18 SEPT 95
Solvent	B7	Conc.	E7
		Test End Date	22 SEPT 95
		Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.30	7.30	7.30	7.15	
Control		7.20	7.10	7.30	7.10
Highest Concentration		6.70	6.60	7.00	6.90

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LIM

Total Number

Mortality Record

Malformation Record

FETAX Control

2 : 80

X 100 =

3%

8 : 78

X 100 =

10.3%

Solvent Control

:

X 100 =

:

X 100 =

Control Length (mm) 8.705

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

0.5 MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	25	0.5	T-test
LOEL	N.A.	N.A.	T-test
LC50	26.155	EC50	5.497
95% CL	25.118	—	27.236
		95% Confidence limits	2.959 — 10.214

Test Teratogenic Index (TI = LC50/EC50):

4.76

95% Confidence limits

2.56

—

8.85

WZ King 28 Dec

BANTLE
Diethylene Glycol w/o MAS #1

Chemical Code:	NH		
Compound:	Diethylene Glycol	Test No.:	2
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:	111-46-6	Laboratory:	OSU / Bantle
Lot No.:	54H0249	Start Date:	18-Jul-95
Glass / Plastic	Plastic	End Date:	22-Jul-95
Microsome lot No.:	J5	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	5	9
FETAX Controls	0	9
MAS Controls	0	8

Cyclophosphamide

Positive Control	100	—
Negative Control	0	18

Results

Without the Metabolic Activation System

LC50	42.43.00	EC50	17.20
95% CI	39.91 - 46.33	95% CI	15.45 - 19.15
Control length	0.99080 cm	TI	2.50
MCIG	19 mg/mL	95% CI	

With the Metabolic Activation System

LC50	34.36	EC50	14.39
95% CI	22.39 - 52.73	95% CI	12.98 - 15.94
Control length	0.94551 cm	TI	2.40 2.39
MCIG	< 11 mg/mL	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

Chemical Code:	NH		
Compound:	Diethylene Glycol	Test No.:	3
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:	111-46-6	Laboratory:	OSU / Bantle
Lot No.:	54H0249	Start Date:	21-Jul-95
Glass / Plastic	Plastic	End Date:	25-Jul-95
Microsome lot No.:	M11	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	1.3	7.5
FETAX Controls	1.3	3.8
MAS Controls	7.5	10.8

Cyclophosphamide

Positive Control	100	—
Negative Control	0	15

Results

Without the Metabolic Activation System

LC50	45.36	EC50	18.09
95% CI	42.53 - 48.37	95% CI	11.61 - 28.17
Control length	1.01074 cm	TI	2.51
MCIG	24 mg/mL	95% CI	

With the Metabolic Activation System

LC50	36.01	EC50	17.32
95% CI	33.06 - 39.23	95% CI	15.85 - 18.94
Control length	0.94106 cm	TI	2.08
MCIG	24 mg/mL	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

Bantle

Diethylene Glycol w/o MAS # 8

Chemical Code:	NH	Test No.:	4
Compound:	Diethylene Glycol	Investigator:	Mendi A. Hull
Source:	Sigma	Laboratory:	OSU / Bantle
CAS No.:	111-46-6	Start Date:	21-Jul-95
Lot No.:	54H0249	End Date:	25-Jul-95
Glass / Plastic	Plastic	Test Units:	mg/mL
Microsome lot No.:	B3		

	% Mortality	% Malformation
FETAX AB Controls	1.3	5.1
FETAX Controls	1.3	7.6
MAS Controls	2.5	5.1

Cyclophosphamide

Positive Control	100	—
Negative Control	5	13

Results

Without the Metabolic Activation System

LC50	31.29	EC50	17.11
95% CI	25.36 - 38.61	95% CI	15.39 - 19.01
Control length	1.01707 cm	TI	1.83
MCIG	13 mg/mL	95% CI	

With the Metabolic Activation System

LC50	18.67	EC50	14.31
95% CI	9.76 - 35.74	95% CI	2.39 - 85.71
Control length	0.73525	TI	1.30
MCIG	>19 mg/mL	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

FETAX - U.S. ARMY ILS PROJECT (OKLA01-RSTS01) RESULTS WITH NH

ENDPOINTS*						
TEST	NO.	MAS	LC50	EC50	TI	MCIG
Range	R	N	20.54 (18.23-23.14)	14.59 (13.52-15.75)	1.41	25.0
Unactivated	1	N	25.19 (24.17-26.26)	16.02 (15.62-16.43)	1.57	10.0
Activated** Fort 1	1	Y	27.30/34.66 (26.51-28.10)/(33.79-35.56)	10.38/10.23 (9.10-11.83)/(9.04-11.57)	2.63/3.39	2.5/2.5
Fort 2	2	Y	24.34/30.72 (22.70-26.10)/(29.42-32.08)	6.65/6.84 (5.95-7.44)/(6.01-7.78)	3.66/4.49	2.5/5.1
Fort 3	3	Y	27.34/32.07 (25.63-29.17)/(30.76-33.43)	8.92/12.15 (7.71-10.31)/(10.67-13.83)	3.07/2.63	2.5/2.5

* Expressed as mg/mL.
** Activated/Unactivated.

FORT
Diethylene Glycol WWO MAST #7
DF

FETAX SUMMARY SHEET

Test No. 1-MAS	
Test Material NH	Investigator F. +
Source ILS	Lab SBL
CAS No.	Lot No.
Composition/Purity	Test Start Date 12/5/94
Solvent	Test End Date 12/9/94
Conc.	Test Units (i.e., mg/ml) mg/mL

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.6	7.7	7.7	7.6	
Control	7.9	7.9	7.9	7.9	
Highest Conc.	7.8	7.8	7.8	7.8	

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>7</u> : <u>80</u> X 100 = <u>8.8</u> %
Solvent Control	<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 mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	<u>2.5</u>	<u>2.5</u> <i>gms</i>

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	NA	5.1 / 5.1	Williams Test
LOEL	NA	8.0 / 8.0	
LC ₅₀ <i>NO MAS</i>	<u>34.66</u> / <u>27.30</u> <i>MA</i>	EC ₅₀ <u>10.23</u> / <u>10.38</u>	
95% Confidence Limits	<u>33.19</u> - <u>35.56</u> <u>26.51</u> - <u>28.10</u>	95% Confidence Limits <u>9.04</u> - <u>11.57</u> <u>9.10</u> - <u>11.83</u>	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			<u>3.39</u> / <u>2.63</u>

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 SUMMARY SHEET

Test No. 2-MAS

Test Material - NH	Investigator Fort
Source ILS	Lab SBL
CAS No.	Lot No.
Composition/Purity	Test Start Date 12/12/94
Solvent	Conc.
	Test End Date 12/16/94
	Test Units (i.e., mg/ml) ml/mL

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.7	7.7	7.7	7.7	
Control	7.9	7.9	7.8	7.9	
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed X 100 = %		
Total Number	2 : 80 X 100 = 2.5%	6 : 78 X 100 = 7.7%
Solvent Control	3 : 40 X 100 = 7.5%	3 : 37 X 100 = 8.1%
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	5.1	2.5

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	16.0 / 8.0	2.5 / 2.5	Williams Test
LOEL	25.2 / 16.0	5.1 / 5.1	
LC ₅₀	30.72 / 24.34	6.84 / 6.65	
95% Confidence limits	24.42 - 32.08 22.70 - 26.10	5.95 - 7.78 5.95 - 7.44	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀) (4.49) / 3.66			

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 = ___ %

FORT
Diethylene Glycol w/ MAS #3

FETAX SUMMARY SHEET

Test No. 3-MAS	
Test Material NH	Investigator Fort
Source ILS	Lab Starr's Assoc.
CAS No.	Lot No.
Test Start Date 1/3/95	
Test End Date 1/7/95	
Composition/Purity	Test Units (i.e., mg/ml) mg/mL
Solvent	Conc.

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.8	7.8	7.8	7.8	
Control	7.9	7.9	7.9	7.9	
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>6</u> : <u>80</u> X 100 = <u>7.5</u> %
Solvent Control MAS	<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 mm	Solvent Control Length mm	
	<u>2.5</u>	<u>2.5</u>
Minimum Concentration to Inhibit Growth (MCIG)		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	MAS NA	8.0 / 5.1	Williams Tests
LOEL	NA	16.0 / 8.0	"
LC ₅₀	<u>32.07</u> / <u>27.34</u>	EC ₅₀ <u>12.15</u> / <u>8.92</u>	
95% Confidence Limits <u>30.76 - 33.43</u> <u>25.63 - 29.17</u>		95% Confidence Limits <u>10.67 - 13.83</u> <u>7.71 - 10.31</u>	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			<u>2.63</u> / <u>3.07</u>

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 = ___ %

PHTHALIC ACID
DATA SUMMARY SHEETS
PHASE III-PART 3

Table 9. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCI_G and TI Values for Phthalic Acid with Coefficients of Variation.

Laboratory	Without MAS*										With MAS																								
	EC50					MCI _G					TI					EC50					MCI _G					TI									
	LC50		CV			Mean (mg/mL)		CV			Mean (mg/mL)		CV			LC50		CV			Mean (mg/mL)		CV			LC50		CV			Mean (mg/mL)		CV		
	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)	Mean (mg/mL)	CV (%)					
	and 95% CI				and 95% CI			and 95% CI			and 95% CI			and 95% CI			and 95% CI			and 95% CI			and 95% CI			and 95% CI			and 95% CI						
1	8.61		8.24		8.00		8.00		1.04		6.98		6.22		1.00		1.83		1.16		1.27		14.5												
	9.24	8.78	3.7	7.57	3.5	7.93	7.54 - 8.31	0.0	1.22	1.11	7.1	3.05	3.34	50.2	3.34	63.6	4.00	0.31 - 3.98	81.3	1.53	1.01 - 1.31														
	8.49	8.33 - 9.23	7.98		8.00		6.00		1.07	1.00 - 1.22	1.78		1.17		0.50																				
2	8.89		8.68		7.30		6.50		1.02		7.59		7.60		5.85		5.70		1.00		1.27		18.4												
	8.10	8.96	8.1	5.83	10.2	7.50	6.90	5.8	1.40	1.22	12.8	6.54	7.32	7.7	5.95	19.7	5.85	3.7	1.24	1.27															
	9.88	7.95 - 9.97	7.99		7.30		7.34		1.24	1.30 - 1.44	7.84		4.99		5.40				1.57	0.95 - 1.59															
3	9.55		4.72		9.46		9.46		2.02		9.07		4.10		9.46		9.46		2.21		1.76		20.4												
	9.47	10.09	8.2	5.64	25.4	4.43	9.46	0.0	1.68	2.51	37.7	8.84	9.21	41	5.08	24.5	9.46	0.0	1.74	1.76															
	11.26	8.95 - 11.74	2.93		9.46		9.46		3.84	1.20 - 1.81	9.73		7.32		9.46				1.33	1.26 - 2.26															

*MAS: Metabolic Activation System

**CI: Confidence Interval

plopdl19

FETAX Summary Sheet

Test No. 218-0021

Test Material	NI w/o MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	22 MAY 95
Solvent	B7	Conc.	E7
		Test End Date	26 MAY 95
		Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.15	6.50	7.20	7.45	
Control		7.00	7.00	7.00	7.05
Highest Concentration		7.20	6.60	7.20	7.48

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Mortality Record

Malformation Record

2 : 80

X 100 = 3%

5 : 78

X 100 = 6.4%

X 100 =

X 100 =

Control Length (mm) 10.014

Solvent Control Length (mm) 120

Minimum Concentration to Inhibit Growth (MCIG) N/A

MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	6	5	T-test
LOEL	N.A.	N.A.	T-test
LC50	8.612	EC50	8.243
95% CL	6.019	--	12.322
		95% Confidence limits	3.434 ---- 19.785

Test Teratogenic Index (TI = LC50/EC50):

1.04 qq

95% Confidence limits

0.41

--

2.69

F.W.C.H.

Phthalic acid w MAS = 1

plopdll9

FETAX Summary Sheet

Test No. 218-002A4

Test Material	NI w/ MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	22 MAY 95
Solvent	B7	Conc.	E7
		Test End Date	26 MAY 95
		Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.15	6.50	7.20	7.45	
Control		7.00	7.00	7.00	7.05
Highest Concentration		7.15	6.70	7.10	6.95

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LIM

Total Number

FETAX Control

Solvent Control

Control Length (mm) 9.816

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

1

MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	4	6	T-test
LOEL	N.A.	N.A.	T-test
LC50	6.978	EC50	6.217
95% CL	6.203	95% Confidence limits	6.217 ---- 6.217
Test Teratogenic Index (TI = LC50/EC50):			1.12 qq
95% Confidence limits			1.00 -- 1.26

plopdl19

FETAX Summary Sheet

Test No. 218-0051

Test Material	NI w/o MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Test Start Date:	14 AUG 95	Test End Date	18 AUG 95
Composition/Purity	C6	Test Units (i.e., mg/ml)	MG/ML
Solvent	B7	Conc.	E7

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	6.5	7	7	7.1	
Control		7	7	7	6.95
Highest Concentration		6.5	7	7.1	7.1

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Mortality Record

Malformation Record

0 : 80

X 100 = 0%

5 : 80

X 100 = 6.3%

:

X 100 =

:

X 100 =

Control Length (mm) 9.885

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

8

MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	8	6	T-test
LOEL	N.A.	N.A.	T-test
LC50	9.239	EC50	7.565
95% CL	7.304	95% Confidence limits	6.161 ---- 9.288

Test Teratogenic Index (TI = LC50/EC50):

1.22 qq

95% Confidence limits

0.89

--

1.67

plopdl19

FETAX Summary Sheet

Test No. 218-005A

Test Material	NTw/ MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	14 AUG 95
Solvent	B7	Conc.	E7
		Test End Date	18 AUG 95
		Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	6.5	7	7	7.1	
Control		7	7	7	6.95
Highest Concentration		6.6	7	6.94	7.06

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length (mm) 8.732

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

4

MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.5	0.5	T-test
LOEL	N.A.	N.A.	T-test
LC50	3.049	EC50	2.631
95% CL	2.184	--	4.255
		95% Confidence limits	1.375 ---- 5.036

Test Teratogenic Index (TI = LC50/EC50):

1.16 qq

95% Confidence limits

0.56

--

2.40

plopdl9

FETAX Summary Sheet

Test No. 218-0061

Test Material	NI w/o MAS			Investigator	DR. FINCH
Source	OSU			Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date:	14 AUG 95
Composition/Purity	C6			Test End Date	18 AUG 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	6.50	7.00	7.00	7.10	
Control		7.00	7.00	7.00	6.95
Highest Concentration		6.50	7.00	7.10	7.10

No. Dead or Malformed X 100 = %		Mortality Record		Malformation Record	
Total Number					
FETAX Control	0 : 80	X 100 = 0%	4 : 80	X 100 = 5.0%	
Solvent Control		X 100 =		X 100 =	
Control Length (mm)	9.662	Solvent Control Length (mm) J20			
Minimum Concentration to Inhibit Growth (MCIG)				8 MG/ML	

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	7	4	T-test
LOEL	N.A.	N.A.	T-test
LC50	8.493	EC50	7.975
95% CL	6.692 -- 10.780	95% Confidence limits	5.812 ---- 10.941

Test Teratogenic Index (TI = LC50/EC50):	1.07 gg
95% Confidence limits	0.72 -- 1.58

FETAX Summary Sheet

Test No. 218-006A

Test Material	NIW/MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	14 AUG 95
Solvent	B7	Test End Date	18 AUG 95
Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	6.50	7.00	7.00	7.10	
Control		7.00	7.00	7.00	6.95
Highest Concentration		6.60	ND	6.96	7.05

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length (mm) 9.468

Minimum Concentration to Inhibit Growth (MCIG)

Mortality Record

Malformation Record

0 : 80

X 100 =

0%

0 : 80

X 100 =

0.0%

X 100 =

X 100 =

Solvent Control Length (mm) J20

0.5 MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	1	N.A.	T-test
LOEL	N.A.	N.A.	T-test
LC50	1.785	EC50	1.165
95% CL	0.000	95% Confidence limits	1.165

Test Teratogenic Index (TI = LC50/EC50):

1.53 qq

95% Confidence limits

0.00

13972720.73

Phthalic acid wa MAS # 1

Chemical Code:	NI		
Compound:	Phthalic Acid	Test No.:	1
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:	877-24-7	Laboratory:	OSU / Bantle
Lot No.:	93H0575	Start Date:	30-Mar-95
Glass / Plastic	Plastic	End Date:	3-Apr-95
Microsome lot No.:	~~AM~~ M12	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	0	2.5
FETAX Controls	0	2.5
MAS Controls	15	32

Cyclophosphamide

Positive Control	100	~~100~~
Negative Control	0	7

Results

Without the Metabolic Activation System

LC50	8.89	EC50	8.68
95% CI		95% CI	7.79 ⁸⁰ = 9.67
Control length	9.4049 mm	TI	1.02
MCIG	none	95% CI	

With the Metabolic Activation System

LC50	7.59	EC50	7.60
95% CI		95% CI	
Control length	9.0737 mm	TI	0.998 1.0
MCIG	5.85	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

FETAX Summary Sheet

BANTLE

Phthalic acid w/o MAS# 2

Chemical Code:	NI		
Compound:	Phthalic Acid	Test No.:	2
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:	877-24-7	Laboratory:	OSU / Bantle
Lot No.:	93H0575	Start Date:	18-Apr-95
Glass / Plastic	Plastic	End Date:	22-Apr-95
Microsome lot No.:	~~A44~~ M12	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	2.5	8.9
FETAX Controls	12.5	5.7
MAS Controls	2.5	10.3

Cyclophosphamide

Positive Control	100	—
Negative Control	0	20.0

Results

Without the Metabolic Activation System

LC50	8.1*	EC50	5.83
95% CI		95% CI	5.43 - 6.25
Control length	0.85662	TI	1.40
MCIG	6.5	95% CI	

* graphing method

With the Metabolic Activation System

LC50	6.54	EC50	5.27
95% CI	5.57 - 7.68	95% CI	4.86 - 5.72
Control length	0.81655	TI	1.24
MCIG	5.85	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

Phthalic acid w w/o MAS # 3

Chemical Code:	NI		
Compound:	Phthalic Acid	Test No.:	3
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:	877-24-7	Laboratory:	OSU / Bantle
Lot No.:	93H0575	Start Date:	24-May-95
Glass / Plastic	Plastic	End Date:	28-May-95
Microsome lot No.:	54	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	10	8
FETAX Controls	13	11.4
MAS Controls	8	10.8

Cyclophosphamide

Positive Control	100	—
Negative Control	8	24

Results

Without the Metabolic Activation System

LC50	9.88*	EC50	7.99
95% CI	9.02 - 10.81	95% CI	7.00 - 9.12
Control length	0.86123 cm	TI	1.24
MCIG	7.3 mg/mL	95% CI	

* did not pass homogeneity

With the Metabolic Activation System

LC50	7.84	EC50	4.99
95% CI	6.83 - 9.01	95% CI	4.59 - 5.43
Control length	0.82652 cm	TI	1.57
MCIG	5.4 µg/mL	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

FETAX - U.S. ARMY ILS PROJECT (OKLA01-RSTS01) RESULTS WITH NI

ENDPOINTS*						
TEST	NO.	MAS	LC50	EC50	TI	MCIG
Range	R	N	7.04 (6.65-7.45)	5.63 (5.16-6.15)	1.25	5.0
Unactivated	1	N	6.84 (6.70-6.97)	6.01 (5.91-6.12)	1.14	6.0
Activated** Fort 1	1	Y	9.07/9.55 (8.73-9.44)(9.23-9.89)	4.10/4.72 (3.61-4.66)(4.22-5.27)	2.21/2.02	9.46/9.46
FORT 2	2	Y	8.84/9.47 (8.37-9.34)(9.47-10.05)	5.64/5.08 (4.56-5.67)(5.11-6.23)	1.74/1.68	9.46/9.46
FORT 3	3	Y	9.73/11.26 (9.32-10.15)(11.13-11.39)	7.32/2.93 (6.91-7.76)(7.24-3.84)	1.33/3.84	9.46/9.46

* Expressed as mg/mL.

** Activated/Unactivated.

FORT
Phthalic acid w/o MAS # 1

FETAX SUMMARY SHEET

Test No. 1-MAS	
Test Material NI	Investigator Fort
Source ILS	Lab Stamm & Assoc.
CAS No.	Lot No.
Test Start Date 1/30/95	
Test End Date 2/3/95	
Composition/Purity	Test Units (i.e., mg/ml) mg/mL
Solvent	Conc.

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
pH					
Stock	4.5 / 7.5	4.5 / 7.5	4.5 / 7.5	4.5 / 7.5	
Control	7.8	7.8	7.8	7.8	
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %		
Solvent Control	0 : 80 X 100 = 0 %	5 : 80 X 100 = 6.25 %
Control Length mm	0 : 40 X 100 = 0 %	1 : 40 X 100 = 2.5 %
Minimum Concentration to Inhibit Growth (MCIG)	9.46	9.46

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	9.55	9.07	
LOEL			
LC ₅₀	9.55	9.07	EC ₅₀ 4.72 / 4.10
95% Confidence Limits	9.23 - 9.89 8.73 - 9.44		95% Confidence Limits 4.22 - 5.27 3.61 - 4.66
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			2.02 / 2.21

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 = _____ %

FORT.
Phthalic acid w/ w/o MAS#

FETAX SUMMARY SHEET

Test No. 2-MAS	
Test Material NI	Investigator Fort
Source ILS	Lab Ston's Assoc.
CAS No. _____	Test Start Date 2/6/95
Lot No. _____	Test End Date 2/10/95
Composition/Purity _____	Test Units (i.e., mg/ml) mg/mL
Solvent _____	Conc. _____

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
pH					
Stock	4.3/7.0	4.3/7.0	4.3/7.0	4.3/7.0	
Control	7.8	7.8	7.8	7.8	
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>6</u> : <u>80</u> X 100 = <u>7.5</u> %
Solvent Control	<u>2</u> : <u>40</u> X 100 = <u>5</u> %	<u>0</u> : <u>38</u> X 100 = <u>0</u> %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	<u>9.46</u>	<u>9.46</u>

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	<u>9.47</u>	<u>8.84</u>	EC ₅₀ <u>5.64</u> / <u>5.08</u>
95% Confidence limits	<u>9.47 - 10.05</u> <u>8.37 - 9.34</u>		95% Confidence Limits <u>5.11 - 6.23</u> <u>4.56 - 5.67</u>
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)		<u>1.68</u>	<u>1.74</u>

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 = ____ %

FORT
Phthalic acid w w/o MAS#

FETAX SUMMARY SHEET

Test No. 3-MAS	
Test Material NI	Investigator Fort / Propst
Source ILS	Lab Starn & Assoc.
CAS No.	Lot No.
Composition/Purity	Test Start Date 2/13/95
Solvent	Test End Date 2/17/95
Conc.	Test Units (i.e., mg/mL) mg/mL

pH	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
Stock	7.2	7.2	7.2	7.2	
Control	7.8	7.8	7.8	7.8	
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %		
	1 : 80 X 100 = 1.3 %	4 : 79 X 100 = 5.1 %
Solvent Control MAS	0 : 40 X 100 = 0 %	1 : 40 X 100 = 2.5 %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	9.46	9.46

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	11.26 / 9.73	EC ₅₀	2.93 / 7.32
95% Confidence limits	11.13 - 11.39 9.32 - 10.15	95% Confidence Limits	2.64 - 3.84 6.91 - 7.76
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)		3.84 / 1.33	

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 = _____ %

DICHLORACETATE
DATA SUMMARY SHEETS
PHASE III-PART 3

plopdl19

FETAX Summary Sheet

Test No. 219-0111

FINCH
Dichloroacetate w/o MAS # 1

Test Material	NJ w/o MAS			Investigator	DR. FINCH
Source	OSU			Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date:	22 JAN 96
Composition/Purity	C6			Test End Date	26 JAN 96
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	6.90	7.75	7.80	7.60	
Control		7.60	7.50	7.30	7.20
Highest Concentration		7.20	7.60	7.70	7.50

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Mortality Record

Malformation Record

0 : 80

X 100 =

0%

5 : 80

X 100 =

6.3%

X 100 =

X 100 =

Control Length (mm) 9.692

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

5 MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used		
NOEL	5	8	T-test		
LOEL	N.A.	N.A.	T-test		
LC50	9.264	EC50	8.375		
95% CL	8.509	--	10.086	95% Confidence limits	8.375 --- 8.375
Test Teratogenic Index (TI = LC50/EC50):			1.11		
95% Confidence limits			1.02	--	1.20

FINCH
Dichloroacetate w MAS #1

plopdl19

FETAX Summary Sheet

Test No. **219-011A2**

Test Material	NJ w/ MAS ¹		Investigator	DR. FINCH
Source	OSU		Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date: 22 JAN 96
Composition/Purity	C6		Test End Date	26 JAN 96
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml) MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	6.90	7.75	7.80	7.60	
Control		7.60	7.50	7.30	7.20
Highest Concentration		6.80	6.60	7.10	N/A

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LIMITS

Total Number

Mortality Record

Malformation Record

FETAX Control

0 : 80 X 100 = 0% 10 : 80 X 100 = 12.5%

Solvent Control

: X 100 = : X 100 =

Control Length (mm) 8.552

Solvent Control length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

NONE

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	N.A.	N.A.	T-test
LOEL	N.A.	N.A.	T-test
LC50	NOT CALCULABLE	EC50	NOT CALCULABLE
95% CL	--	95% Confidence limits	----

Test Teratogenic Index (TI = LC50/EC50): NOT CALCULABLE

95% Confidence limits

FINCH
Dichloroacetate w/o MAS # 2

plopdll9

FETAX Summary Sheet

Test No. 219-0121

Test Material	N/w MAS			Investigator	DR. FINCH
Source	OSU			Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date:	26 FEB 96
Composition/Purity	C6			Test End Date	01 MAR 96
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7.70	7.80	7.60	7.50	
Control		7.10	7.40	7.45	7.40
Highest Concentration		7.50	7.70	7.70	7.55

No. Dead or Malformed				
X 100 = %				
Total Number	Mortality Record		Malformation Record	
FETAX Control	0 : 80	X 100 = 0%	3 : 80	X 100 = 3.8%
Solvent Control	:	X 100 =	:	X 100 =
Control Length (mm)	10.321	Solvent Control Length (mm) J20		
Minimum Concentration to Inhibit Growth (MCIG)				5 MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	8.75	8.75	T-test
LOEL	N.A.	N.A.	T-test
LC50	9.519	EC50	9.060
95% CL	8.505	-- 10.655	95% Confidence limits 8.727 --- 9.405
Test Teratogenic Index (TI = LC50/EC50):			1.05
95% Confidence limits			0.93 -- 1.18

FINCH
Dichloroacetate w MAS F 2

plopdl19

FETAX Summary Sheet

Test No. ***219-012A***

Test Material	NJ w/ MAS ²		Investigator	DR. FINCH
Source	OSU		Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date: 26 FEB 96
Composition/Purity	C6		Test End Date	01 MAR 96
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml) MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.70	7.80	7.60	7.50	
Control		7.10	7.40	7.45	7.40
Highest Concentration		7.30	7.30	7.30	6.60

No. Dead or Malformed				
X 100 = %				
Total Number	Mortality Record		Malformation Record	
FETAX Control	0 : 80	X 100 = 0%	2 : 80	X 100 = 2.5%
Solvent Control	:	X 100 =	:	X 100 =
Control Length (mm)	9.982	Solvent Control Length (mm) J20		
Minimum Concentration to Inhibit Growth (MCIG)				5 MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	5	N.A.	T-test
LOEL	N.A.	N.A.	T-test
LC50	5.289	EC50	4.072
95% CL	4.944 -- 5.657	95% Confidence limits	1.316 --- 12.596
Test Teratogenic Index (TI = LC50/EC50):		1.30	
95% Confidence limits		0.42 -- 4.03	

FETAX Summary Sheet

Test No. 216-0091

Test Material	- New w/o MAS			Investigator	DR. FINCH
Source	OSU			Laboratory	USABRD
CAS No.	B4	Lot No.	E5	Test Start Date:	13 NOV 95
Composition/Purity	C6			Test End Date	17 NOV 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

	Day 0	Day 1	Day 2	Day 3	Day 4
pH Stock	7.00	7.40	7.60	7.60	
Control		7.40	6.95	7.10	7.40
Highest Concentration		7.60	7.40	7.55	7.70

No. Dead or Malformed		MALFORMATION EXCEED ASTM LIMITS			
X 100 = %					
Total Number	Mortality Record			Malformation Record	
	1 : 80	X 100 = 1%	8 : 79	X 100 = 10.1%	
	:	X 100 =	:	X 100 =	
FETAX Control					
Solvent Control					
Control Length (mm) 9.889		Solvent Control Length (mm) J20			
Minimum Concentration to Inhibit Growth (MCIG)		5.75 MG/ML			

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	5.75	N.A.	T-test
LOEL	N.A.	N.A.	T-test
LC50	8.874	EC50	7.139
95% CL	8.697	-- 9.054	95% Confidence limits 6.426 ---- 7.930

Test Teratogenic Index (TI = LC50/EC50):	1.24 qq
95% Confidence limits	1.12 -- 1.38

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FETAX Summary Sheet

Test No. 219-007A?

Test Material	NJ w/ MAS			Investigator	DR. FINCH
Source	OSU			Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date:	13 NOV 95
Composition/Purity	C6			Test End Date	17 NOV 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.00	7.40	7.60	7.60	
Control		7.40	6.95	7.10	7.40
Highest Concentration		6.60	6.70	6.65	6.60

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LIMITS

Total Number

Mortality Record

Malformation Record

FETAX Control

2 : 80

X 100 = 3%

6 : 78 X 100 = 7.7%

Solvent Control

:

X 100 =

X 100 =

Control Length (mm) 9.484

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

NONE MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	N.A.	N.A.	T-test
LOEL	N.A.	N.A.	T-test
LC50	NOT CALCULABLE	EC50 NOT CALCULABLE	
95% CL	--	95% Confidence limits	----

Test Teratogenic Index (TI = LC50/EC50):

95% Confidence limits

Chemical Code:	NJ	Test No.:	1
Compound:	Dichloroacetic Acid	Investigator:	Mendi A. Hull
Source:	Sigma	Laboratory:	OSU / Bantle
CAS No.:	79-43-6	Start Date:	25-Oct-95
Lot No.:	25H3432	End Date:	29-Oct-95
Glass / Plastic	Plastic	Test Units:	mg/mL
Microsome lot No.:	B5		

	% Mortality	% Malformation
FETAX AB Controls	1.25	3.75
FETAX Controls	1.25	8.86
MAS Controls	7.5	10.5

Cyclophosphamide

Positive Control	100	—
Negative Control	0	14.5

Results

Without the Metabolic Activation System

LC50	6.7	EC50	2.34
95% CI		95% CI	1.87 - 2.91
Control length	0.90267 cm	TI	2.86
MCIG	2.80 mg/mL	95% CI	

With the Metabolic Activation System

LC50	6.3	EC50	0.8 0.90
95% CI		95% CI	0.74 - 1.09
Control length	0.87012 cm	TI	7.00
MCIG	1.65 mg/mL	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

Chemical Code:	NJ		
Compound:	Dichloroacetic Acid	Test No.:	2
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:	79-43-6	Laboratory:	OSU / Bantle
Lot No.:	25H3432	Start Date:	25-Oct-95
Glass / Plastic	Plastic	End Date:	29-Oct-95
Microsome lot No.:	B6	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	12.5	2.5
FETAX Controls	0	1.25
MAS Controls	0	5

Cyclophosphamide

Positive Control	100	-
Negative Control	10	8.3

Results

Without the Metabolic Activation System

LC50	6.42	EC50	2.11
95% CI	0.88049 cm	95% CI	1.41 - 3.15
Control length	0.88049 cm	TI	3.04
MCIG	6.25 mg/mL	95% CI	

With the Metabolic Activation System

LC50	6.2	EC50	0.92
95% CI		95% CI	0.70 - 1.21
Control length	0.89779 cm	TI	6.74
MCIG	2.8 mg/mL	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

Chemical Code:	NJ	Test No.:	45
Compound:	Dichloroacetic Acid	Investigator:	Mendi A. Hull
Source:	Sigma	Laboratory:	OSU / Bantle
CAS No.:	79-43-6	Start Date:	18-Dec-95
Lot No.:	25H3432	End Date:	22-Dec-95
Glass / Plastic	Plastic	Test Units:	mg/mL
Microsome lot No.:	M16		

	% Mortality	% Malformation
FETAX AB Controls	10.0	11.1
FETAX Controls	13.7	15.9
MAS Controls	10.0	11.1

Cyclophosphamide

Positive Control	100	—
Negative Control	35	30.7

Results

Without the Metabolic Activation System

LC50	6.58	EC50	1.19
95% CI	4.91 - 8.82	95% CI	0.76 - 1.86
Control length	1.03466 cm	TI	5.53
MCIG	0.2 mg/mL	95% CI	

With the Metabolic Activation System

LC50	3.01	EC50	0.79
95% CI	1.56 - 5.80	95% CI	0.57 - 1.09
Control length	0.93802	TI	3.81
MCIG	8.57 mg/mL	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

FETAX - U.S. ARMY ILS PROJECT (OKLA01-RSTS01) RESULTS WITH NJ

TEST	NO.	MAS	ENDPOINTS*			
			LC50	EC50	TI	MCIG
Range	R	N	1.94 (1.59-2.35)	0.79 (0.60-1.04)	2.46	5.0
Unactivated	1	N	4.69 (4.25-5.17)	3.07 (2.83-3.31)	1.53	5.0
Activated** FORT 1	1	Y	6.51/6.50 (6.34-6.69)(6.33-6.68)	3.94/4.28 (3.65-4.26/3.99-4.59)	1.65/1.52	5.83/5.83
FORT 2	2	Y	6.59/6.92 (6.26-6.95)(6.65-7.21)	4.77/4.90 (4.53-5.03)(4.62-5.20)	1.38/1.41	5.83/5.83
FORT 3	3	Y	6.71/7.66 (6.57-6.85)(7.41-7.92)	4.28/6.27 (3.89-4.71)(5.59-7.03)	1.57/1.22	7.88/7.88

*Expressed as mg/mL.

** Activated/Unactivated.

FORT
Dichloroacetate w/wo MAS # 1

FETAX SUMMARY SHEET

Test Material <u>NJ</u>		Test No. <u>1-MAS</u>	
Source <u>ILS</u>		Investigator <u>Fort</u>	
CAS No.	Lot No.	Lab <u>Stam's Assoc</u>	
Composition/Purity		Test Start Date <u>1/30/95</u>	
Solvent		Test End Date <u>2/3/95</u>	
Conc.		Test Units (i.e., mg/mL) <u>mg/mL</u>	

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	<u>1.7/7.0</u>	<u>1.8/7.0</u>	<u>1.7/7.0</u>	<u>1.7/7.0</u>	
Control	<u>7.8</u>	<u>7.8</u>	<u>7.8</u>	<u>7.8</u>	
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed X 100 = %		
Total Number	<u>0 : 80 X 100 = 0 %</u>	<u>4 : 80 X 100 = 5 %</u>
Solvent Control	<u>0 : 40 X 100 = 0 %</u>	<u>1 : 40 X 100 = 5 %</u>
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG) <u>5.83 / 5.83</u> <u>QAB 7/12/95</u>		

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	<u>6.50 / 6.51</u>	EC ₅₀ <u>4.28 / 3.94</u>	
95% Confidence limits	<u>6.33 - 6.68</u> <u>6.34 - 6.67</u>	95% Confidence Limits <u>3.99 - 4.57</u> <u>3.65 - 4.26</u>	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)		<u>1.52 / 1.65</u>	

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 = ____ %

FORT
Dichloroacetic Acid w/w/o MAS # 2

FETAX SUMMARY SHEET

Test No. 2-MAS

Test Material <u>NJ</u>		Investigator <u>Fort</u>
Source <u>ILS</u>		Laboratory <u>Starn & Assoc.</u>
CAS No.	Lot No.	Test Start Date <u>2/6/95</u>
Composition/Purity		Test End Date <u>2/10/95</u>
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	<u>1.5/7.0</u>	<u>1.6/1.0</u>	<u>1.5/7.0</u>	<u>1.5/7.0</u>	
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>0 : 40</u> X 100 = <u>0</u> %	<u>1 : 40</u> X 100 = <u>2.5</u> %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	<u>5.83</u>	<u>5.83</u> <u>2/12/95</u>

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	<u>6.92</u> <u>6.59</u>	EC ₅₀ <u>4.90</u> <u>4.77</u>	
95% Confidence Limits <u>6.65 - 7.21</u> <u>6.26 - 6.95</u>		95% Confidence Limits <u>4.62 - 5.20</u> <u>4.52 - 5.03</u>	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)		<u>1.41</u> <u>1.38</u>	

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 = ____ %

FORT
 Nicholas and W. C. MAS#

FETAX SUMMARY SHEET

Test No. 3-MAS	
Test Material NJ	Investigator Probst / Fort
Source LS	Lab Starn's Assoc.
CAS No.	Lot No.
Composition/Purity	Test Start Date 2/13/95
Solvent	Conc.
	Test End Date 2/17/95
	Test Units (i.e., mg/mL) mg/mL

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.1	7.1	7.1	7.1	
Control	7.8	7.8	7.8	7.8	
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number X 100 = %		
	1 : 80 X 100 = 7.5%	3 : 78 X 100 = 3.8%
Solvent Control	0 : 40 X 100 = 0%	2 : 40 X 100 = 5%
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	7.88	7.88

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	7.66 / 6.71	EC ₅₀	6.27 / 4.28
95% Confidence Limits	7.41 - 7.92 6.57 - 6.85	95% Confidence Limits	5.59 - 7.03 3.84 - 4.71
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀) 1.22 / 1.57			

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 = ___ %

SODIUM BROMATE
DATA SUMMARY SHEETS
PHASE III-PART 3

Table 11. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCIG and TI Values for Sodium Bromate with Coefficients of Variation.

Laboratory	Without MAS*										With MAS										
	LC50					EC50					MCIG					TI					
	Mean (mg/mL)		CV (%)	Mean (mg/mL)		CV (%)	Mean (mg/mL)		CV (%)	Mean (mg/mL)		CV (%)	Mean (mg/mL)		CV (%)	Mean (mg/mL)		CV (%)	Mean (mg/mL)		CV (%)
	and 95% CI**			and 95% CI		and 95% CI		and 95% CI		and 95% CI		and 95% CI		and 95% CI		and 95% CI		and 95% CI			
1	1.34		0.28		0.10		0.22		0.62		0.05		0.27		0.05		0.36		0.36		
	1.16	1.21	7.6	0.58	0.41	31.3	0.25	0.20	35.4	1.99	3.29	33.9	0.15	0.11	91.9	0.05	1.48	1.21	50.9		
	1.13	1.08 - 1.33	0.36	0.23 - 0.38	0.25	0.10 - 0.30	0.25	0.10 - 0.30	0.15	0.13 - 0.22	0.08	-0.07 - 0.61	0.15	0.08	0.10	0.03 - 0.10	1.80	0.36 - 2.07			
2	1.05		0.32		0.50		0.75		0.29		0.21		0.27		0.21		2.59				
	1.35	1.18	10.5	0.28	0.29	7.4	0.16	0.27	58.6	4.82	4.12	15.4	1.16	0.28	8.0	0.16	12.2	4.14	3.20	21.0	
	1.15	1.01 - 1.36	0.27	0.26 - 0.32	0.16	0.05 - 0.30	0.16	0.05 - 0.30	0.09	0.58 - 1.16	0.24	0.24 - 0.30	0.09	0.24	0.21	0.16 - 0.23	2.88	2.27 - 4.14			
3	2.12		0.49		1.30		0.72		0.20		0.57		0.20		0.57		3.60				
	1.29	1.35	45.3	0.46	0.39	32.5	1.30	1.19	13.5	2.80	3.37	20.0	0.31	0.15	24.0	0.42	41.2	2.07	2.44	34.5	
	0.63	0.30 - 2.19	0.21	0.21 - 0.56	0.36	0.96 - 1.41	0.36	0.96 - 1.41	0.18	0.08 - 0.72	0.11	0.10 - 0.20	0.18	0.11	0.18	0.17 - 0.61	1.64	1.27 - 2.60			

*MAS= Metabolic Activation System

**CI= Confidence Interval

plopdl9.

FETAX Summary Sheet

Test No. 221-0011

Test Material	NL w/o MAS	Investigator	DR. FINCH
Source	QSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	22 AUG 95
Solvent	B7	Conc.	E7
		Test End Date	26 AUG 95
		Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.25	7.2	7.4	7.3	
Control		6.9	6.95	7.05	7
Highest Concentration		7.2	7.2	7.4	7.3

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length (mm) 10.308

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

0.1

MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	1	0.1	T-test
LOEL	N.A.	N.A.	T-test
LC50	1.335	EC50 0.284	
95% CL	1.226	-- 1.454	95% Confidence limits 0.159 ---- 0.506
Test Teratogenic Index (TI = LC50/EC50):			4.71 qq
95% Confidence limits			2.62 -- 8.45

F.N.C.

No Brute w MAS # 1

plopdl19

FETAX Summary Sheet

Test No. 221-001A

Test Material	NL w/ MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Test Start Date:	22 AUG 95	Test End Date	26 AUG 95
Composition/Purity	C6	Test Units (i.e., mg/ml)	MG/ML
Solvent	B7	Conc.	E7

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.25	7.2	7.4	7.3	
Control		6.9	6.95	7.05	7
Highest Concentration		6.95	7.01	6.86	6.99

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Mortality Record

Malformation Record

0 : 80 X 100 = 0%

4 : 80 X 100 = 5.0%

X 100 =

X 100 =

Control Length (mm) 9.46

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG) 0.05

MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.1	0.05	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.224	EC50	0.620
95% CL	0.176	--	0.284
95% Confidence limits	0.097	----	3.982
Test Teratogenic Index (TI = LC50/EC50):			
95% Confidence limits	0.06	--	2.35
			0.36 qq

plopdl19

FETAX Summary Sheet

Test No. 221-0021

Test Material	NL w/o MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	11 SEPT. 95
Solvent	B7	Conc.	E7
		Test End Date	15 SEPT 95
		Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.50	7.50	7.25	7.25	
Control		7.00	7.10	7.00	6.95
Highest Concentration		7.40	7.20	7.20	7.20

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Mortality Record

Malformation Record

0 : 80

X 100 = 0%

5 : 80

X 100 = 6.3%

X 100 =

X 100 =

Control Length (mm) 10.454

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

0.25 MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.5	0.1	T-test
LOEL	N.A.	N.A.	T-test
LC50	1.159	EC50	0.583
95% CL	0.879	--	1.528
		95% Confidence limits	0.313 ---- 1.086

Test Teratogenic Index (TI = LC50/EC50):

1.99 qq

95% Confidence limits

1.01

--

3.93

FINCH
Na Bromate w MAS # 2

plod119

FETAX Summary Sheet

Test No. 221-002A

Test Material	NL w/ MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Test Start Date:	11 SEPT. 95		
Composition/Purity	C6	Test End Date	15 SEPT 95
Solvent	B7	Conc.	E7
Test Units (i.e., mg/ml)	MG/ML		

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.50	7.50	7.25	7.25	
Control		7.00	7.10	7.00	6.95
Highest Concentration		7.26	6.60	6.90	6.70

No. Dead or Malformed
X 100 = %
Total Number
FETAX Control
Solvent Control

MALFORMATION EXCEED ASTM LIMIT

Mortality Record			Malformation Record		
0	80	X 100 = 0%	8	80	X 100 = 10.0%
		X 100 =			X 100 =

Control Length (mm)	9.471	Solvent Control Length (mm) J20	
Minimum Concentration to Inhibit Growth (MCIG)	0.05 MG/ML		

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.05	N.A.	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.154	EC50	0.105
95% CL	0.082	95% Confidence limits	0.029 --- 0.377

qq

Test Teratogenic Index (TI = LC50/EC50):	1.48 qq
95% Confidence limits	0.35 --- 6.17

qq

plopdl9

FETAX Summary Sheet

Test No. **421-0036**

Test Material	NE w/o MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	11 SPT 95
Solvent	B7	Conc.	E7
		Test End Date	15 SPT 95
		Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.50	7.50	7.25	7.25	
Control		7.00	7.10	7.00	6.95
Highest Concentration		7.40	7.20	7.20	7.20

No. Dead or Malformed
X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length (mm) 9.847

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

0.25 MG/M

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.25	0.1	T-test
LOEL	N.A.	N.A.	T-test
LC50	1.125	EC50	0.356
95% CL	0.948	95% Confidence limits	0.243 ---- 0.523
Test Teratogenic Index (TI = LC50/EC50):			3.16
95% Confidence limits			2.07 -- 4.81

plopdl19

FINCH
Na Bromate w MAS #3

FETAX Summary Sheet

Test No. **221003A7**

Test Material	NEW/MAS1			Investigator	DR. FINCH
Source	OSU			Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date:	11 SPT 95
Composition/Purity	C6			Test End Date	15 SPT 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.50	7.50	7.25	7.25	
Control		7.00	7.10	7.00	6.95
Highest Concentration		6.95	6.70	6.90	ND

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LIM

Total Number

Mortality Record

Malformation Record

FETAX Control

2 : 80

X 100 = 3%

14 : 78

X 100 =

17.9%

Solvent Control

X 100 =

X 100 =

Control Length (mm) 8.857

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

0.1 MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	N.A.	0.1	T-test
LOEL	N.A.	N.A.	T-test
LC50	0.150	EC50	0.084
95% CL	0.118	-- 0.192	95% Confidence limits 0.060 --- 0.116
Test Teratogenic Index (TI = LC50/EC50):			1.80
95% Confidence limits			1.19 -- 2.70

Chemical Code:	NL		
Compound:	Sodium Bromate	Test No.:	1
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:	7789-38-0	Laboratory:	OSU / Bantle
Lot No.:		Start Date:	24-Aug-95
Glass / Plastic	plastic	End Date:	28-Aug-95
Microsome lot No.:	M17	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	9	10.9
FETAX Controls	10	5.5
MAS Controls	12.5	11.4

Cyclophosphamide

Positive Control	100	—
Negative Control	10	16.6

Results

Without the Metabolic Activation System

LC50	1.05	EC50	0.32
95% CI	0.96 - 1.15	95% CI	0.28 - 0.37
Control length	0.84597 cm	TI	3.28
MCIG	0.5	95% CI	

With the Metabolic Activation System

LC50	0.75	EC50	0.29
95% CI	0.68 - 0.83	95% CI	0.21 - 0.39
Control length	0.85043 cm	TI	2.59
MCIG	0.21	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

Na Bromate w/o MAS #2

Chemical Code:	NL		
Compound:	Sodium Bromate	Test No.:	2
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:	7789-38-0	Laboratory:	OSU / Bantle
Lot No.:		Start Date:	30-Aug-95
Glass / Plastic	plastic	End Date:	3-Sep-95
Microsome lot No.:	lot Ocast 1	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	1	8
FETAX Controls	0	13
MAS Controls	0	3

Cyclophosphamide

Positive Control	100	-
Negative Control	8	11

Results

Without the Metabolic Activation System

LC50	1.35	EC50	0.28
95% CI	1.22 - 1.50	95% CI	0.24 - 0.33
Control length	0.85280 cm	TI	4.82
MCIG	0.16	95% CI	

With the Metabolic Activation System

LC50	1.16	EC50	0.28
95% CI	1.08 - 1.25	95% CI	0.24 - 0.33
Control length	0.81853 cm	TI	4.14
MCIG	0.16	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

NaBromate w/o MAS # 3

Chemical Code:	NL		
Compound:	Sodium Bromate	Test No.:	3
Source:	Sigma	Investigator:	Mendi A. Hull
CAS No.:	7789-38-0	Laboratory:	OSU / Bantle
Lot No.:		Start Date:	30-Aug-95
Glass / Plastic	plastic	End Date:	3-Sep-95
Microsome lot No.:	M17	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	5.0	9.2
FETAX Controls	3.75	6.4
MAS Controls	5.0	7.8

Cyclophosphamide

Positive Control	100	—
Negative Control	2.5	7.6

Results

Without the Metabolic Activation System

LC50	1.15	EC50	0.27
95% CI	1.03 - 1.31	95% CI	0.23 - 0.30
Control length	0.11 ^{0.86316}	TI	4.26
MCIG	0.16	95% CI	

With the Metabolic Activation System

LC50	0.69	EC50	0.24
95% CI	0.62 - 0.76	95% CI	0.22 - 0.27
Control length	0.76132	TI	2.88
MCIG	0.21	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

FETAX - U.S. ARMY ILS PROJECT (OKLA01-RSTS01) RESULTS WITH NL

ENDPOINTS*						
TEST	NO.	MAS	LC50	EC50	TI	MCIG
Range	R	N	0.79 0.68-0.92	0.71 ND	1.11	1.0
Unactivated	1	N	1.30 1.22-1.39	0.57 0.52-0.61	2.28	1.0
Activated** Fort 1	1	Y	0.72/2.12 (0.67-0.78)(2.02-2.23)	0.20/0.49 (0.17-0.23)(0.44-0.55)	3.60/4.32	0.57/1.3
Fort 2	2	Y	0.31/1.29 (0.27-0.36)(1.19-1.39)	0.15/0.46 (0.13-0.17)(0.41-0.52)	2.07/2.80	0.42/1.3
Fort 3	3	Y	0.18/0.63 (0.15-0.20)(0.54-0.74)	0.11/0.21 (0.10-0.12)(0.18-0.24)	1.64/3.00	0.18/0.96

* Expressed as mg/ml.

** Activated/Unactivated.

FETAX SUMMARY SHEET

FORT

Na Bromate w/o MAS #1

Test No. 1 - MAS

Test Material	NL	Investigator	Fort
Source	ELS	Lab	Starn & Assoc.
CAS No.		Lot No.	
Composition/Purity		Test Start Date	3/6/95
Solvent		Test End Date	3/10/95
	Conc.	Test Units (i.e., mg/ml)	mg/mL

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.8	7.8	7.8	7.8	
Control	7.9	7.9	7.9	7.8	
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed Total Number X 100 = %		
	0 : 80 X 100 = %	1 : 80 X 100 = 1.3 %
Solvent Control MAS	3 : 40 X 100 = 7.5 %	3 : 37 X 100 = %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	1.3 / 0.57	7/17/95

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	2.12 / 0.72	EC ₅₀ 0.49 / 0.20	
95% Confidence limits	2.02 - 2.23 0.67 - 0.78	95% Confidence Limits 0.44 - 0.55 0.17 - 0.23	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			4.32 / 3.60

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 SUMMARY SHEET

FOR: Na Bromate wwb MAS # 2

Test No. 2-MAS

Test Material	NL	Investigator	F. A. T.
Source	ILS	Lab	Stam & Assoc.
CAS No.		Test Start Date	3/13/95
Composition/Purity		Test End Date	3/17/95
Solvent		Conc.	
		Test Units (i.e., mg/ml)	mg/mL

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.7	7.8	7.8	7.8	
Control	7.9	7.9	7.9	7.8	
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number	0 : 80 X 100 = 0 %	5 : 80 X 100 = 6.25 %
Solvent Control	0 : 40 X 100 = 0 %	3 : 40 X 100 = 7.5 %
Control Length		
Minimum Concentration to Inhibit Growth (MCIG)	1.3	0.42

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	1.29	0.31	EC ₅₀ 0.46
95% Confidence limits	0.27 - 1.39	0.13 - 0.52	95% Confidence Limits 0.13 - 0.17
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			2.80

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L		
2500 mg/L		

FETAX SUMMARY SHEET

FORT
Na Bromate w/0 MAS #3

Test No. 3-MAS

Test Material	NL	Investigator	FORT
Source	ILS	Laboratory	Stover & Assoc.
CAS No.		Lot No.	
Composition/Purity		Test Start Date	3/21/95
Solvent		Conc.	
		Test End Date	3/25/95
		Test Units (i.e., mg/ml)	mg/mL

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.8	7.7	7.7	7.8	
Control	7.8	7.8	7.8	7.8	
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number		
	0 : 0 X 100 = 0%	7 : 80 X 100 = 8.75%
Solvent Control	2 : 40 X 100 = 5%	3 : 40 X 100 = 7.5%
Control Length	mm	mm
Minimum Concentration to Inhibit Growth (MCIG)	0.96	0.18

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL	NA	0.13/0.06	Williams Test
LOEL	NA	0.18/0.13	
LC ₅₀	0.63/0.18	EC ₅₀ 0.21/0.11	
95% Confidence Limits	0.54 - 0.74 0.15 - 0.20	95% Confidence Limits 0.18 - 0.24 0.10 - 0.12	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			
3.06/1.64			

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 = _____ %

TRIBROMOACETIC ACID
DATA SUMMARY SHEETS
PHASE III-PART 3

Table 12. Intralaboratory Mean 96-hr LC50, EC50 (malformation), MCIg and TI Values for Tribromoacetic Acid with Coefficients of Variation.

Laboratory	Without MAS*										With MAS									
	LC50					EC50					LC50					EC50				
	Mean (mg/mL) and 95% CI**	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	#NDM ^b	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	#NDM ^b	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)	Mean (mg/mL) and 95% CI	CV (%)
1	15.60	5.92	5.00	5.00	5.00	2.03	2.03	6.29	3.54	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
	14.58	15.59	5.3	9.27	18.4	18.4	18.4	7.97	7.97	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
	10.59	14.45 - 16.73	8.72	5.94	10.00	5.00	5.00	5.94	10.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
2	16.44	5.00	8.39	8.39	8.39	3.29	3.29	7.15	3.07	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38	0.38
	17.90	15.87	12.1	5.06	4.27	25.3	25.3	4.27	4.27	5.57	5.57	5.57	5.57	5.57	5.57	5.57	5.57	5.57	5.57	5.57
	13.28	13.20 - 18.55	2.74	2.77	5.76	5.57	5.57	2.77	5.76	5.57	5.57	5.57	5.57	5.57	5.57	5.57	5.57	5.57	5.57	5.57
3	9.10	1.7	6.09	6.09	6.09	5.35	5.35	10.34	0.83	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
	8.96	9.08	1.0	1.82	1.63	11.7	11.7	1.63	1.63	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
	9.19	8.95 - 9.21	1.37	1.37	1.89	6.71	6.71	1.37	1.89	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09

*MAS: Metabolic Activation System

**CI: Confidence Interval

FINCH
Tribromoacetic acid w MAS #1

plopdl9

FETAX Summary Sheet

Test No. 222-001A

Test Material	NM w/ MAS		Investigator	DR. FINCH
Source	OSU		Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date: 11 SEPT 95
Composition/Purity	C6			Test End Date 15 SEPT 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml) MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.30	7.40	7.00	6.80	
Control		7.00	7.10	7.00	6.95
Highest Concentration		7.10	6.80	7.00	6.60

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LIM

Total Number

FETAX Control

Solvent Control

Control Length (mm) 9.357

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

5 MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	1	1	T-test
LOEL	N.A.	N.A.	T-test
LC50	6.287	EC50	3.544
qq	5.482	95% Confidence limits	0.161 --- 77.853

Test Teratogenic Index (TI = LC50/EC50):

1.77 qq

95% Confidence limits

0.08

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39.08

plopdl9

FETAX Summary Sheet

Test No. 222-0031

Test Material	NM w/o MAS	Investigator	DR. FINCH
Source	OSU	Laboratory	USABRDL
CAS No.	B4	Lot No.	E5
Composition/Purity	C6	Test Start Date:	2 OCT 95
Solvent	B7	Conc.	E7
		Test End Date	6 OCT 95
		Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.30	7.30	7.20	7.55	
Control		7.10	7.20	7.40	7.50
Highest Concentration		7.60	7.70	7.50	7.40

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LIM

Total Number

Mortality Record

Malformation Record

FETAX Control

0 : 80

X 100 = 0%

8 : 80

X 100 = 10.0%

Solvent Control

X 100 =

X 100 =

Control Length (mm) 9.618

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

5 MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	0.5	5	T-test
LOEL	N.A.	N.A.	T-test
LC50	14.581	EC50	9.273
95% CL	12.958	--	16.408
		95% Confidence limits	7.320 ---- 11.747

Test Teratogenic Index (TI = LC50/EC50):

1.57 qq

95% Confidence limits

1.21

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2.05

dpd119

FETAX Summary Sheet

FINCH
Tribromoacetic acid w MAS # 2

Test No. 222-003A

Test Material	NM w/ MAS			Investigator	DR. FINCH
Source	OSU			Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date:	2 OCT 95
Composition/Purity	C6			Test End Date	6 OCT 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.30	7.30	7.20	7.55	
Control		7.10	7.20	7.40	7.50
Highest Concentration		7.15	7.00	7.10	6.90

No. Dead or Malformed

X 100 = %

MALFORMATION EXCEED ASTM LIMIT

Total Number

FETAX Control

Solvent Control

Control Length (mm) 9.096

Minimum Concentration to Inhibit Growth (MCIG)

Mortality Record

Malformation Record

0 : 80

X 100 = 0%

6 : 80

X 100 = 7.5%

X 100 =

X 100 =

Solvent Control Length (mm) J20

5 MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	N.A.	7.5	T-test
LOEL	N.A.	N.A.	T-test
qq	7.627	EC50	7.393
95% CL	4.730	--	12.298
		95% Confidence limits	4.604 ---- 11.872
Test Teratogenic Index (TI = LC50/EC50):		1.03 qq	
95% Confidence limits		0.53	-- 2.02

FINCH

Tribromoacetic acid w/o MAS # 6

plopdl19

FETAX Summary Sheet

Test No. ~~1227-0045~~

Test Material	NM w/o MAS		Investigator	DR. FINCH	
Source	OSU		Laboratory	USABRDL	
CAS No.	B4	Lot No.	E5	Test Start Date:	13 NOV 95
Composition/Purity	C6		Test End Date	17 NOV 95	
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml)	MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.00	7.55	7.70	7.45	
Control		7.40	6.95	7.10	7.40
Highest Concentration		7.75	7.40	7.50	7.60

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length (mm) 9.723

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

5 MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	5	1	T-test
LOEL	N.A.	N.A.	T-test
LC50	16.589	EC50	8.719
95% CL	15.464	95% Confidence limits	6.960 ---- 10.921

Test Teratogenic Index (TI = LC50/EC50):

95% Confidence limits

1.50

1.90 qq

2.41

* There are 24 instead of 20 embryos in the first Fetax-AB control.

plopdl19

FETAX Summary Sheet

Test No. **2228014A7**

Test Material	NM w/ MAS?		Investigator	DR. FINCH
Source	OSU		Laboratory	USABRDL
CAS No.	B4	Lot No.	E5	Test Start Date: 13 NOV 95
Composition/Purity	C6		Test End Date	17 NOV 95
Solvent	B7	Conc.	E7	Test Units (i.e., mg/ml) MG/ML

pH

	Day 0	Day 1	Day 2	Day 3	Day 4
Stock	7.00	7.55	7.70	7.45	
Control		7.40	6.95	7.10	7.40
Highest Concentration		6.85	6.70	6.70	6.70

No. Dead or Malformed

X 100 = %

Total Number

FETAX Control

Solvent Control

Control Length (mm) 9.982

Solvent Control Length (mm) J20

Minimum Concentration to Inhibit Growth (MCIG)

1 MG/ML

Test Material/Compound Results

Test	Mortality	Malformation	Statistical Test Used
NOEL	1	5	T-test
LOEL	N.A.	N.A.	T-test
LC50	13.947	EC50	10.773
95% CL	12.829	--	15.163
		95% Confidence limits	9.002 ---- 12.892

Test Teratogenic Index (TI = LC50/EC50):

1.29 qq

95% Confidence limits

1.06

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1.58

Chemical Code:	NM		
Compound:	Tribromoacetic Acid	Test No.:	Test 3
Source:	Aldrich-Sigma	Investigator:	Mendi A. Hull
CAS No.:	7789-38-0	Laboratory:	OSU / Bantle
Lot No.:	JG07905 KF.	Start Date:	12-Oct-95
Glass / Plastic	plastic	End Date:	16-Oct-95
Microsome lot No.:		Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	2.5	7.6
FETAX Controls	5.0	3.9
MAS Controls	2.5	7.7

Cyclophosphamide

Positive Control	100	-
Negative Control	5.0	13.2

Results

Without the Metabolic Activation System

LC50	16.44	EC50	5.00
95% CI	11.84 - 22.83	95% CI	4.24 - 5.39
Control length	1.0148 cm	TI	3.29
MCIG	8.39 mg/mL	95% CI	

With the Metabolic Activation System

LC50	7.15	EC50	3.07
95% CI	3.06 - 16.75	95% CI	2.23 - 4.31
Control length	0.97956 cm	TI	2.33
MCIG	0.38 mg/mL	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

Tribromoacetic acid w/o MAS #2

Chemical Code:	NM		
Compound:	Tribromoacetic Acid	Test No.:	Test 4
Source:	Aldrich-Sigma	Investigator:	Mendi A. Hull
CAS No.:	7789-38-0	Laboratory:	OSU / Bantle
Lot No.:	JG07905 KF	Start Date:	20-Oct-95
Glass / Plastic	plastic	End Date:	24-Oct-95
Microsome lot No.:	B5	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	1.25	6.25
FETAX Controls	2.5	3.75
MAS Controls	2.5 0	2.5

Cyclophosphamide

Positive Control	100	—
Negative Control	7.5	10.8

Results

Without the Metabolic Activation System

LC50	17.90	EC50	5.06
95% CI	15.15 - 21.15	95% CI	4.33 - 5.92
Control length	0.97933 cm	TI	3.54
MCIG	8.39 mg/mL	95% CI	—

With the Metabolic Activation System

LC50	17.72	EC50	4.47
95% CI	9.80 - 32.02	95% CI	3.25 - 6.15
Control length	0.94603	TI	3.96
MCIG	5.57 mg/mL	95% CI	—

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

Chemical Code:	NM		
Compound:	Tribromoacetic Acid	Test No.:	Test 5
Source:	Aldrich ~~Sigma~~	Investigator:	Mendi A. Hull
CAS No.:	7789-38-0	Laboratory:	OSU / Bantle
Lot No.:	JG07905KF	Start Date:	8-Nov-95
Glass / Plastic	plastic	End Date:	12-Nov-95
Microsome lot No.:	B06 *	Test Units:	mg/mL

	% Mortality	% Malformation
FETAX AB Controls	3.75	6.50
FETAX Controls	16.25	8.90
MAS Controls	12.50	8.60

Cyclophosphamide

Positive Control	100	—
Negative Control	2.5	17.9

Results

Without the Metabolic Activation System

LC50	13.28	EC50	2.74
95% CI	10.38-16.99	95% CI	2.24-3.35
Control length	0.98259 cm	TI	4.85
MCIG	5.57 mg/mL	95% CI	

With the Metabolic Activation System

LC50	9.39	EC50	2.12
95% CI	7.24-12.17	95% CI	1.52-2.96
Control length	0.98126 cm	TI	4.43
MCIG	10.76 mg/mL	95% CI	

FETAX AB = FETAX antibiotic solution

MAS = Metabolic Activation System

CI = Confidence Interval

FETAX - U.S. ARMY ILS PROJECT (OKLA01-RSTS01) RESULTS WITH NM

ENDPOINTS*						
TEST	NO.	MAS	LC50	EC50	T1	MCIG
Range	R	N	14.30 13.13-15.55	1.75 1.43-2.15		>10.0
Unactivated	1	N	19.04 18.44-19.66	2.00 1.87-2.14	9.52	18.0
Activated** <i>Fort 1</i>	1	Y	10.34/9.10 (9.78-10.93)(8.21-10.08)	0.83/1.70 (0.64-1.09)(1.44-2.01)	12.46/5.35	>6.09/>6.09
<i>Fort 2</i>	2	Y	6.56/8.86 (5.70-7.54)(8.07-9.95)	1.12/1.82 (0.90-1.39)(1.60-2.07)	5.86/4.92	6.09/>6.09
<i>Fort 3</i>	3	Y	5.24/9.19 (4.48-6.12)(8.25-10.12)	1.26/1.37 (0.97-1.63)(1.10-1.71)	4.16/6.71	3.80/>6.09

* Expressed as mg/ml.

** Activated/Unactivated.

FORT
Tribromoacetate add w w6 MAS# 1
FETAX SUMMARY SHEET

Test Material <u>NIM</u>		Investigator <u>Fort</u>
Source <u>ILS</u>	Lab <u>Storier Assoc.</u>	
CAS No.	Lot No.	Test Start Date <u>3/13/95</u>
Composition/Purity		Test End Date <u>3/17/95</u>
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	<u>1.5 / 7.0</u>	<u>1.5 / 7.0</u>	<u>1.5 / 7.0</u>	<u>1.5 / 7.0</u>	
Control	<u>7.8</u>	<u>7.8</u>	<u>7.8</u>	<u>7.8</u>	
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number	<u>0 : 80</u> X 100 = <u>0</u> %	<u>6 : 80</u> X 100 = <u>0</u> %
Solvent Control	<u>0 : 80</u> X 100 = <u>0</u> %	<u>3 : 40</u> X 100 = <u>7.5</u> %
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	<u>> 6.09</u>	<u>> 6.09</u> <i>9/11/95</i>

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	<u>9.10 / 10.34</u>	EC ₅₀ <u>1.70 / 0.83</u>	
95% Confidence limits	<u>8.24 - 10.08</u> <u>9.78 - 10.93</u>	95% Confidence Limits <u>1.44 - 2.01</u> <u>0.64 - 1.09</u>	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			<u>5.35 / 12.46</u>

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 SUMMARY SHEET

Test No. 2-MAS

Test Material	NM	Investigator	Fort
Source	ILS	LAD	Stover & Assoc.
CAS No.		Test Start Date	3/3/95
Lot No.		Test End Date	3/7/95
Composition/Purity		Test Units (i.e., mg/mL)	mg/mL
Solvent		Conc.	

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.2	7.2	7.2	7.2	
Control	7.8	7.8	7.8	7.8	
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD *
No. Dead or Malformed		
Total Number	X 100 = %	X 100 = %
Solvent Control	1 : 80 X 100 = 1.3 %	7 : 79 X 100 = 8.9 %
Control Length mm		
Minimum Concentration to Inhibit Growth (MCIG)	76.09	76.00

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	8.96	6.56	EC ₅₀ 1.82 / 1.12
95% Confidence limits	8.07 - 1.95 5.70 - 7.54	95% Confidence Limits	1.60 - 2.09 0.90 - 1.39
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			4.92 / 5.86

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 SUMMARY SHEET

FCRT

Tribromoacetic acid w/wo MAS# 3

Test No. 3 - MAS

Test Material	NM	Investigator	Fort
Source	ILS	Lab	Storn's Assoc.
CAS No.		Lot No.	
Composition/Purity		Test Start Date	4/3/95
Solvent		Test End Date	4/7/95
Conc.		Test Units (i.e., mg/ml)	mg/mL

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
— pH —					
Stock	7.3	7.3	7.3	7.3	
Control	7.9	7.9	7.9	7.9	
Highest Conc.					

FETAX CONTROL	MORTALITY RECORD	MALFORMATION RECORD
No. Dead or Malformed		
Total Number		
	$2 : 80 \times 100 = 0\%$	$6 : 80 \times 100 = 7.5\%$
Solvent Control	$1 : 40 \times 100 = 2.5\%$	$3 : 39 \times 100 = \_\_\%$
Control Length mm	Solvent Control Length mm	
Minimum Concentration to Inhibit Growth (MCIG)	76.09	3.8 g/L

TEST MATERIAL/COMPOUND : RESULTS

TEST	MORTALITY	MALFORMATION	STATISTICAL TEST USED
NOEL			
LOEL			
LC ₅₀	9.19	5.24	EC ₅₀ 1.37
95% Confidence limits	8.35 - 10.12 4.48 - 6.12	95% Confidence Limits 1.18 - 1.71 0.97 - 1.63	
TEST TERATOGENIC INDEX (TI = LC ₅₀ / EC ₅₀)			6.71 / 4.16

POSITIVE CONTROL: 6 AMINONICOTINAMIDE (6-AN) RESULTS

CONCENTRATION	MORTALITY	MALFORMATION
5.5 mg/L	$\_\_\_\_\_\_ : \_\_\_\_\_\_ \times 100 = \_\_\_\_\_\_%$	$\_\_\_\_\_\_ : \_\_\_\_\_\_ \times 100 = \_\_\_\_\_\_%$
2500 mg/L	$\_\_\_\_\_\_ : \_\_\_\_\_\_ \times 100 = \_\_\_\_\_\_%$	$\_\_\_\_\_\_ : \_\_\_\_\_\_ \times 100 = \_\_\_\_\_\_%$

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Publications:

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