3.0. CHARACTERIZATION OF SUBSTANCES TESTED IN FETAX

FETAX test data from 276 studies involving 137 substances, not including environmental samples, were located, reviewed, extracted, and entered into the NICEATM FETAX database (**Appendix 2** contains substances tested without metabolic activation, **Appendix 3** contains substances tested with metabolic activation). Sources for these data included peer-reviewed literature (including studies accepted for publication) and non peer-reviewed book chapters. Excluded from consideration was information provided in abstracts, manuscripts not accepted for publication, publications that did not provide quantitative data, studies conducted where the test substances were not identified, and studies conducted that did not follow the general FETAX protocol described in the ASTM FETAX Guideline (1991, 1998).

3.1 Rationale for Chemicals/Products Selected for FETAX Validation Studies

Only limited information is available on the selection rationale for the chemicals/products tested in the five FETAX validation studies. It does not appear that selection was based on testing substances that represented a range of chemical or product classes. Rather, selection appeared to have been based primarily on the availability of prior FETAX test results and laboratory mammal teratological data. Specific chemical selection rationale for each FETAX validation study is presented by individual validation study.

Validation Study Phase I was classified as a training and protocol evaluation phase (Bantle et al., 1994a). 6-AN, hydroxyurea, and isoniazid were selected for testing without metabolic activation based on their positive performance in previous FETAX studies (Bantle et al., 1994a).

In Phase II (Bantle et al., 1994b), caffeine, 5-fluorouracil, saccharin, and sodium cyclamate were tested without metabolic activation. These test substances were selected for testing without metabolic activation based on their negative (saccharin, sodium cyclamate) and positive (caffeine, 5-fluorouracil) performance in previous FETAX studies (Bantle et al., 1994b).

Validation Study Phase III.1 (Bantle et al., 1996) involved the testing, without metabolic activation, of β-aminopropionitrile, ascorbic acid, copper sulfate, monosodium glutamate, sodium acetate, and sodium arsenate. The rationale for selecting these test substances was not provided in the validation report. Three of the six test substances (ascorbic acid, sodium acetate, copper sulfate) had been tested previously in FETAX. In laboratory mammals, ascorbic acid, monosodium glutamate, and sodium acetate are non-teratogenic, while sodium arsenate and copper sulfate are teratogenic.

The purpose of Validation Study Phase III.2 (Fort et al., 1998) was to conduct an inter-laboratory validation of an exogenous MAS developed for use with FETAX. Caffeine and CP were tested, with and without metabolic activation, and were selected based on their activation profiles. CP is efficiently bioactivated by P-450 to reactive metabolites, while the addition of metabolic activation was not anticipated to significantly alter the response of *X. laevis* to caffeine. CP is a human and laboratory mammal teratogen; caffeine is a teratogen in laboratory mammals but not humans.

Validation Study Phase III.3 involved the testing, with and without metabolic activation, of 12 substances (acrylamide, boric acid, dichloroacetate, diethylene glycol, ethylene glycol, glycerol, phthalic acid, sodium arsenite, sodium bromate, sodium iodoacetate, tribromoacetic acid, and triethylene glycol dimethyl ether) (Bantle et al., 1999). The rationale for the selection of the test substances was not provided in the validation report. However, it is likely that selection was based on the availability of relevant laboratory mammal data and the suitability of the test substance for testing in FETAX (e.g., water solubility, lack of volatility). Of the 12 substances tested, Bantle et al. (1999) reported that seven (boric acid, dichloroacetate, sodium arsenite, sodium bromate, sodium iodoacetate, tribromoacetic acid, triethylene glycol dimethylether) were classified as teratogens in laboratory mammals, two (glycerol, phthalic acid) were classified as non-teratogens in laboratory mammals, and three (ascorbic acid, sodium acetate, copper sulfate) were classified as equivocal with respect to laboratory mammal teratogenicity (i.e., were not consistently positive in all laboratory mammal species tested).

3.2 Rationale for the Numbers of Chemicals/Products Tested in FETAX

A rationale for the numbers of chemicals/products tested in each of the five validation studies was not provided. However, the most likely basis was the extent of available funding.

3.3 Description of Chemical and Product Classes Evaluated in FETAX

Information on chemical and product classes for substances tested in FETAX are provided in **Appendix 1**; the most common chemical and product classes are provided in **Tables 1a** and **1b**, respectively. Substances were assigned to chemical classes based on available information from standardized references (e.g., *The Merck Index* [Budavari, 1996]) and from an assessment of chemical structure by an organic chemist. The most numerically prevalent chemical classes were

Table 1a. Major Chemical Classes Evaluated with FETAX

Major Chemical Classes	Number of Chemicals
Alcohols (including glycols)	22
Amides	16
Amides and Hydrazides	29*
Amines	19*
Halogenated Organic Compounds	12
Esters	12
Heavy Metals	14
Hydrazides and Hydrazines	14
Nitrogen Heterocyclic Compounds	40*
Organic (Phenolic and Carboxylic) Acids	24*
Salts	20
Total	260

*Classes indicated had adequate comparative data (i.e., at least 15 chemicals with FETAX and either laboratory mammal or human study results) to warrant an assessment of performance (**Section 6**).

Table 1b. Major Product Classes Evaluated with FETAX

Major Product Classes	Number of Products
Antimicrobials	5
Chemical Synthesis	17
Cosmetics	6
Dyes	7
Food Additives	11
Fossil Fuels	6
Pesticides	13
Pharmaceuticals	45*
Photographic Chemicals	5
Polymers	6
Total	121

*Classes indicated had adequate comparative data (i.e., at least 15 chemicals with both FETAX and either laboratory mammal or human study results) to warrant an assessment of performance (**Section 6**)

alcohols (including glycols); amides; amines; halogenated organic compounds; esters; heavy metals and their salts; hydrazides and hydrazines; nitrogen heterocyclic compounds; organic (phenolic and carboxylic) acids; and salts. Of the 137 substances tested in FETAX, 8 substances were not classified within these chemical classes, 67 substances were included in one chemical class, 41 substances were included in two chemical classes, 15 substances were included in three chemical classes, three substances were included in four chemical classes, two substances were included in five chemical classes, and one substances was included in six chemical classes.

Product classes were assigned based primarily on ChemFinder and *The Merck Index.* The most common product classes tested in FETAX were antimicrobials, chemical synthesis, cosmetics, dyes, food additives, fossil fuels, pesticides, pharmaceuticals, photographic chemicals, and polymers (including monomers). Of the 137 substances tested in FETAX, 63 substances were not classified within these product classes, 50 substances were included in one product class, 14

substances were included in two product classes, seven substances were included in three product classes, and three substances were included in four product classes.

3.4 Coding Used in FETAX Validation Studies

Coded chemicals were not used in the Phase I Validation Study (Bantle et al., 1994a), but were used in the Phase II (Bantle et al., 1994b), Phase III.1 (Bantle et al., 1996), Phase III.2 (Fort et al., 1998), and Phase III.3 (Bantle et al., 1999) Validation Studies.

3.5 FETAX-Tested Substances in the Smith et al. (1983) List of Candidate Substances/Conditions for *In Vitro* Teratogenesis Test Validation

In 1983, Smith et al. published a list of candidate substances/conditions for *in vitro* teratogenesis test validation. NICEATM identified the number of Smith list substances evaluated in FETAX, with or without metabolic activation (**Table 2**). NICEATM also identified those substances listed by Smith et al. (1983) that might be expected to require metabolic activation before a teratogenic response would be induced. This identification was based on whether the substance was positive in one or more *in vitro* genetic toxicological tests (generally the *Salmonella typhimurium* reverse mutation assay) with, but not without, metabolic activation. *In vitro* genetic toxicology data were obtained from the EPA Genetic Activity Profile (GAP) database (www.epa.gov/gapdb/) and the NTP Salmonella test database. This method for identifying substances that may require metabolic activation to be teratogenic *in vitro* assumes a common mechanism between mutagenicity and teratogenicity that may not be valid. Of the 47 substances listed, 26 substances (55%) were tested in FETAX without metabolic activation, while nine of these 26 substances (19% of the total list) were tested also with metabolic activation. Of the nine substances tested with metabolic activation, relevant *in vitro* genetic toxicology data were located for seven. Two of these seven substances potentially require metabolic activation to be teratogenic.

Table 2. Smith et al. (1983) Suggested List of Substances/Conditions for *In Vitro* Teratogenesis Testing

Substance	Tested in FETAX	
	Without Activation	**With Activation**
Acetozolamide	Not Tested	Not Tested
Amaranth	**Tested**	Not Tested
6-Aminonicotinamide	**Tested**	Not Tested
Aspirin	Not Tested	Not Tested
Caffeine*	**Tested**	**Tested**
Carbon tetrachloride*	Not Tested	Not Tested
Chlorambucil**	Not Tested	Not Tested
Coumarin*	**Tested**	Not Tested
Cyclophosphamide**	**Tested**	**Tested**
Cytochalasin D*	**Tested**	**Tested**
Dexamethasone	Not Tested	Not Tested
Diazapam*	**Tested**	Not Tested
Diethylstilbestrol*	Not Tested	Not Tested
Dilantin	**Tested**	**Tested**
Diphenylhydramine HCl	**Tested**	Not Tested
Doxylamine succinate*	**Tested**	**Tested**
EM12	Not Tested	Not Tested
Ethyl alcohol*	**Tested**	Not Tested
Ethylenethiourea*	Not Tested	Not Tested
N-Ethyl-N-nitrosourea*	**Tested**	**Tested**
5-Fluorouracil*	**Tested**	Not Tested
Formaldehyde*	Not Tested	Not Tested
Hexahydrophthalimide glutarimide	Not Tested	Not Tested
Hydroxyurea*	**Tested**	Not Tested
Hyperthermia	Not Tested	Not Tested

Table 2. Smith et al. (1983) Suggested List of Substances/Conditions for *In Vitro* Teratogenesis Testing (Continued)

Substance	Tested in FETAX	
	Without Activation	**With Activation**
Isoniazid*	**Tested**	**Tested**
Meprobamate	Not Tested	Not Tested
Methotrexate*	**Tested**	Not Tested
Methyl mercury chloride*	**Tested**	Not Tested
Mirex	Not Tested	Not Tested
Nitrilotriacetate*	**Tested**	Not Tested
Penicillin G	Not Tested	Not Tested
L-Phenylalanine	Not Tested	Not Tested
Phthalimide	Not Tested	Not Tested
Procarbazine*	**Tested**	Not Tested
Retenoic acid (all trans)*	**Tested**	Not Tested
Retinoic acid –13 cis*	**Tested**	Not Tested
Saccharin*	**Tested**	Not Tested
Sodium arsenate*	**Tested**	Not Tested
Sodium cyclamate*	**Tested**	Not Tested
Testosterone proprionate	Not Tested	Not Tested
Thalidomide	Not Tested	Not Tested
Trichloroethylene*	**Tested**	**Tested**
Trichlorophenoxyacetic acid*	Not Tested	Not Tested
Urethane**	**Tested**	**Tested**
Vincristine sulfate*	Not Tested	Not Tested
Vinyl chloride**	Not Tested	Not Tested

Bolded chemical names indicate substances tested in FETAX without and/or with MAS.

* or ** indicates chemicals that do not or do appear to require metabolic activation, respectively, to induce a positive response in an *in vitro* genetic toxicological test according to the EPA Genetic Activity Profile (GAP) database (www.epa.gov/gapdb/) and the NTP Salmonella test database.

3.6 Section 3 Conclusions

In the five FETAX validation studies, it appears that selection rationale for the substances tested was based primarily on the availability of prior FETAX test results and laboratory mammal teratological data rather than on selecting materials with relevant mammal/human data that represented a range of chemical or product classes. A rationale for the numbers of substances tested in each of the five validation studies was not provided. The most likely explanation is the level of available funding. Coded substances were used in all but the first of five validation studies. However, in the Phase II Validation study, all laboratories used the same preset test substance concentrations. If additional validation studies are considered for FETAX, more substances on the Smith et al. list or an updated list should be considered for inclusion. Also, consideration should be given to the role of metabolic activation in *in vitro* teratogenicity studies, and in the identification of appropriate substances to test with metabolic activation.