

## **Appendix F**

### **Reference Substance Information**

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## **Appendix F1**

### **NRU Test Information for the 72 Reference Substances**

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**Table F-1 NRU Test Information for the 72 Reference Substances**

<b>Chemical</b>	<b>CASRN</b>	<b>Purity (%)</b>	<b>Supplier</b>	<b>pH in 3T3 Medium<sup>a</sup></b>	<b>Concentrations Tested in 3T3 Assay (µg/mL)</b>	<b>pH in NHK Medium<sup>b</sup></b>	<b>Concentrations Tested in NHK Assay (µg/mL)</b>
Acetaminophen	103-90-2	99	Sigma	8.1	4.7-1000	7.7	11.8-4000
Acetonitrile	75-05-8	99.5	Sigma	8.4	118-100000	7.9	8.12-200000
Acetylsalicylic acid	50-78-2	99.5	Sigma	7.5	9.4-2500	6.9	11.8-2500
Aminopterin	54-62-6	100.3	Fluka	8.1	0.00005-0.1	7.2	67.4-1000
5-Aminosalicylic acid	89-57-6	99	Sigma	6.7	169-2500	7.5	2.4-500
Amitriptyline HCl	549-18-8	100	Sigma	8.1	0.4-100	7.6	0.24-100
Arsenic III trioxide	1327-53-3	99.9	Sigma	7.9	0.169-100	7.5	0.46-100
Atropine sulfate monohydrate	5908-99-6	100	Fluka	7.9	4.7-1000	7.5	3.8-10000
Boric acid	10043-35-3	101.1	Fluka	7.1	4.7-10000	7.4	28.3-10000
Busulfan	55-98-1	100.2	Fluka	8.1	2.4-500	7.8	2.35-800
Cadmium II chloride	10108-64-2	99.8	Fluka	8.1	0.135-5	7.7	0.337-100
Caffeine	58-08-2	99.9	Fluka	8.3	1.6-5000	7.8	3.25-10000
Carbamazepine	298-46-4	> 99	Sigma	8.0	0.3-1000	7.9	1.88-1000
Carbon tetrachloride	56-23-5	> 99.5	Sigma-Aldrich	NA	169-7000	7.7	11.8-7000
Chloral hydrate	302-17-0	100.1	Sigma	8.4	4.7-1000	7.6	4.7-1000
Chloramphenicol	56-75-7	> 99	Fluka	8.3	4.7-2500	7.8	9.15-2500
Citric acid	77-92-9	98	Sigma	2.9	23.5-10000	4.0	23.5-10000

**Table F-1 NRU Test Information for the 72 Reference Substances**

Chemical	CASRN	Purity (%)	Supplier	pH in 3T3 Medium <sup>a</sup>	Concentrations Tested in 3T3 Assay (µg/mL)	pH in NHK Medium <sup>b</sup>	Concentrations Tested in NHK Assay (µg/mL)
Colchicine	64-86-8	> 98	Fluka	8.2	0	7.7	0.0014-0.10
Cupric sulfate pentahydrate	7758-99-8	99.7	Sigma	7.8	0.0059-5.0	7.4	2.4-750
Cycloheximide	66-81-9	100	Sigma	8.0	0.01-50	7.8	0.0040-100
Dibutyl phthalate	84-74-2	> 99	Sigma	8.0	3.7-2500	7.7	0.9-1000
Dichlorvos	62-73-7	99.5	Chem Service, Inc.	8.1	0.5-100	7.7	0.235-500
Diethyl phthalate	84-66-2	99.5	Aldrich	8.1	4.7-2000	7.8	2.35-2000
Digoxin	20830-75-5	98.6	Sigma	8.2	3.5-1000	7.8	0.0000047-0.100
Dimethylformamide	68-12-2	99.95	Sigma-Aldrich	8.1	236-50000	7.7	70.6-30000
Diquat dibromide monohydrate	6385-62-2	99	Chem Service, Inc.	7.9	0.03-100	7.7	0.47-500
Disulfoton	298-04-4	99.4	Chem Service, Inc.	8.0	2.4-2500	7.8	2.4-2500
Endosulfan	115-29-7	99.5	Chem Service, Inc.	8.3	0.1-100	7.8	0.67-50
Epinephrine bitartrate	51-42-3	> 99	Sigma-Aldrich	7.9	6.74-200	7.6	4.7-1000
Ethanol	64-17-5	100	Sigma-Aldrich	8.6	1011-50000	7.8	118-150000
Ethylene glycol	107-21-1	99.99	Sigma	8.4	1770-100000	7.8	1770-100000
Fenpropathrin	39515-41-8	91.8	Valent	8.3	2.4-500	7.8	0.301-100
Gibberellic acid	77-06-5	99	Acros	4.5	1348-100000	6.5	23.6-10000
Glutethimide	77-21-4	> 99	Sigma-Aldrich	8.0	19-1000	7.7	4.7-1000

**Table F-1 NRU Test Information for the 72 Reference Substances**

<b>Chemical</b>	<b>CASRN</b>	<b>Purity (%)</b>	<b>Supplier</b>	<b>pH in 3T3 Medium<sup>a</sup></b>	<b>Concentrations Tested in 3T3 Assay (µg/mL)</b>	<b>pH in NHK Medium<sup>b</sup></b>	<b>Concentrations Tested in NHK Assay (µg/mL)</b>
Glycerol	56-81-5	99.9	Sigma	8.2	4586-100000	7.8	47-101960
Haloperidol	52-86-8	99	Sigma	8.3	0.1-25	7.7	0.188-100
Hexachlorophene	70-30-4	99.2	Sigma-Aldrich	8.1	0.5-100	7.5	0.002-1
Lactic acid	50-21-5	88.6	Sigma	3.2	47.1-10000	3.0	47.1-10000
Lindane	58-89-9	100	Sigma	8.1	0.8-2500	7.7	2.35-2000
Lithium I carbonate	554-13-2	99.4	Sigma	9.3	74.3-1102.5	9.5	4.7-2000
Meprobamate	57-53-4	> 99	Sigma	8.1	9.4-2500	7.7	4.71-2500
Mercury II chloride	7487-94-7	99.5	Sigma	8.1	0.05-10	7.6	0.67-10
Methanol	67-56-1	99.97	Sigma-Aldrich	8.0	398-3500 (no toxicity)	7.6	9.42-2500
Nicotine	54-11-5	> 99.0	Fluka	8.8	94.9-1000	8.5	8.02-5000
Paraquat	1910-42-5	100	Sigma	7.9	0.5-100	7.8	2.4-1000
Parathion	56-38-2	98	Supelco	8.2	0.5-2500	7.7	0.47-1500
Phenobarbital	50-06-6	100	Spectrum	7.7	11.8-2500	7.4	7.06-3000
Phenol	108-95-2	> 99	Sigma	8.0	0.3-1500	7.7	4.7-1000
Phenylthiourea	103-85-5	98	Sigma	8.1	0.8-2500	7.7	9.42-2500
Physostigmine	57-47-6	100	Sigma	8.1	5.4-200	7.7	0.32-1000
Potassium I chloride	7447-40-7	100	Sigma	8.3	163-15000	7.8	23.5-10000

**Table F-1 NRU Test Information for the 72 Reference Substances**

<b>Chemical</b>	<b>CASRN</b>	<b>Purity (%)</b>	<b>Supplier</b>	<b>pH in 3T3 Medium<sup>a</sup></b>	<b>Concentrations Tested in 3T3 Assay (µg/mL)</b>	<b>pH in NHK Medium<sup>b</sup></b>	<b>Concentrations Tested in NHK Assay (µg/mL)</b>
Potassium cyanide	151-50-8	99.4	Mallinckrodt Baker	9.0	0.5-1500	8.2	0.401-500
Procainamide HCl	51-06-9	99.7	Sigma-Aldrich	8.3	67-1000	7.5	47-10000
2-Propanol	67-63-0	> 99.9	Sigma	8.5	1011-50000	7.7	47.1-20000
Propranolol HCl	3506-09-0	100	Sigma	7.9	1.78-1000	7.4	1.8-350
Propylparaben	94-13-3	> 99	Fluka	8.1	2.4-1000	7.7	0.47-300
Sodium arsenite	7784-46-5	> 99.0	Fluka	8.0	0.05-10.0	7.7	0.038-30
Sodium chloride	7647-14-5	99.5	Sigma	8.2	94-20000	7.9	4.71-10000
Sodium dichromate dihydrate	7789-12-0	100.4	Sigma	8.0	0.03-10.0	7.7	0.0318-100
Sodium I fluoride	7681-49-4	100	Sigma	8.1	10.1-1000	7.7	0.3-1000
Sodium hypochlorite	7681-52-9	12.9% Cl	Sigma-Aldrich	8.0	24-10000	7.7	47.1-10000
Sodium oxalate	62-76-0	99.99	Sigma-Aldrich	8.1	1.2-500	7.7	40.5-2000
Sodium selenate	13413-01-0	100	Sigma-Aldrich	8.2	6.8-300	7.8	0.47-556
Strychnine	57-24-9	99	Sigma	8.4	9.5-800	7.8	1.18-500
Thallium I sulfate	7446-18-6	99.995	Aldrich	8.3	0.1-500	7.8	0.0047-2
Trichloroacetic acid	76-03-9	> 99	Aldrich	2.3	24-10000	1.9	33.0-10000
1,1,1-Trichloroethane	71-55-6	99.78	Sigma-Aldrich	8.4	1686-50000	8.0	674-10000
Triethylenemelamine	51-18-3	98	Acros	8.0	0.02-4	7.6	0.024-10

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<b>Chemical</b>	<b>CASRN</b>	<b>Purity (%)</b>	<b>Supplier</b>	<b>pH in 3T3 Medium<sup>a</sup></b>	<b>Concentrations Tested in 3T3 Assay (µg/mL)</b>	<b>pH in NHK Medium<sup>b</sup></b>	<b>Concentrations Tested in NHK Assay (µg/mL)</b>
Triphenyltin hydroxide	76-87-9	~ 99.5	Sigma-Aldrich	8.0	0.0002-0.1	7.6	0.005-0.1
Valproic acid	99-66-1	100	Sigma	6.9	12-2500	6.0	11.8-2500
Verapamil HCl	152-11-4	98	Sigma-Aldrich	8.1	3.4-100	7.5	3.8-1500
Xylene	1330-20-7	99.9	Mallinckrodt Baker	6.8	398-2500	7.5	190-2000

Abbreviations:NRU=Neutral red uptake; CASRN=Chemical Abstracts Service Registry Number; 3T3=BALB/c 3T3 fibroblasts; NHK=Normal human epidermal keratinocytes; pH=Mean pH of the highest concentration tested (of all acceptable NRU tests)

<sup>a</sup>3T3 Medium - Dulbecco's Modification of Eagle's Medium, with supplements.

<sup>b</sup>NHK medium - Keratinocyte Growth Medium (KGM® from Cambrex).

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## **Appendix F2**

### **Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances**

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**Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances**

Chemical	CASRN	LD <sub>50</sub> (mg/kg) <sup>a</sup>	MW (g/mol)	Chemical Class <sup>b</sup>	Water Solubility <sup>c</sup>	pK <sup>d</sup>	log Kow <sup>e</sup>	Boiling Point (°C) <sup>d</sup>	Toxic Effect Class <sup>f</sup>	Passage of Blood: Brain Barrier <sup>g</sup>	Metabolic Activation/ Inactivation <sup>f</sup>	Mechanism of Lethality <sup>f</sup>
Acetaminophen	103-90-2	2404	151.20	Organic compound; Amide	Slightly in cold, much more in hot; 1-5 mg/mL @ 22°C	NA	0.8	NA	Liver toxin	Free?	More toxic intracellular metabolites	Covalent NAPQI binding and lipid peroxidation.
Acetonitrile	75-05-8	3798	41.05	Organic compound; Nitrile	Miscible; ≥100 mg/mL @ 22.5°C	-4.30	-0.34	81.6	CNS stimulant	Presumed	Must be metabolized to hydrogen cyanide for effect.	Assumed to be same as cyanide: General enzyme inhibition. High affinity for Fe <sup>+++</sup> . Inhibits cell respiration by inhibition of cytochrome oxidase; solvent
Acetylsalicylic acid	50-78-2	1000	180.20	Organic compound; Carboxylic acid; Phenol	3.3 mg/mL @ 25°C; 4.6 mg/mL @ 25°C; <1 mg/mL @ 23°C	3.49@ 25°C	1.19	NA	Gastric irritant, CNS (encephalo- pathy), kidney toxin	Restricted	Salicylic acid is an active metabolite	General cell poison, works by uncoupling oxidation phosphorylation and inhibition of Kreb's cycle dehydrogenases.
Aminopterin	54-62-6	3 (mouse)	476.45	Organic compound; Heterocyclic compound	NA	5.5	NA	NA	Hematotoxin	Presumed to be minimal (like methotrexate)	Not expected to require metabolism for toxicity	Hypothetical: Inhibits folic acid utilization and thus cell proliferation.
5-Aminosalicylic acid	89-57-6	7749 (mouse)	153.10	Organic compound; Carboxylic acid; Phenol	2 mg/mL; <1 mg/mL @ 21°C	3.25	1.32	NA	Kidney toxin	Yes	Not activated	Unknown
Amitriptyline HCl	549-18-8	319	313.90	Organic compound; Polycyclic compound	0.0097 mg/mL @ 24°C/HCl is freely soluble	9.4	5.04	NA	Cardiotoxin	Free	Nortriptyline, a metabolite, also active	Hypothetical: Blocks norepinephrine, 5- hydroxytryptamine, and dopamine presynaptic uptake; prevents reuptake of heart norepinephrine.

**Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances**

Chemical	CASRN	LD <sub>50</sub> (mg/kg) <sup>a</sup>	MW (g/mol)	Chemical Class <sup>b</sup>	Water Solubility <sup>c</sup>	pK <sup>d</sup>	log Kow <sup>e</sup>	Boiling Point (°C) <sup>d</sup>	Toxic Effect Class <sup>f</sup>	Passage of Blood: Brain Barrier <sup>g</sup>	Metabolic Activation/Inactivation <sup>f</sup>	Mechanism of Lethality <sup>f</sup>
Arsenic III trioxide	1327-53-3	20	197.80	Inorganic compound; Arsenical	sparingly in cold; in 15 parts boiling; 17 mg/mL @ 16°C	NA	NA	465	CNS toxin (encephalopathy)	Restricted	No	Cellular poison. Multisystem failure due to uncoupling oxidative phosphorylation & inhibition of pyruvate and succinate oxidative pathways; Apoptosis induction; angiogenesis inhibition; cellular growth inhibition
Atropine sulfate monohydrate	5908-99-6	623	694.80	Organic compound; Heterocyclic compound	2.2 mg/mL	NA	1.83	NA	CNS stimulant	Free	No	Antimuscarinic, anticholinergic action. Competitive antagonism of anticholinesterase at cardiac & CNS receptor sites.
Boric acid	10043-35-3	2660	61.83	Inorganic compound; Boron compound; Acids	56 mg/mL in cold water; 10-50 mg/mL @ 19°C	NA	NA	300	Skin, kidney, liver, testicular toxin	Yes	No	Inhibits enzymes involved in metabolism and RNA synthesis. <sup>g</sup>
Busulfan	55-98-1	2	246.31	Organic compound; Alcohol; Acyclic hydrocarbon; Sulfur compound	Decomposes	NA	-0.52	NA	Hematotoxin	Freely (similar to plasma concentration) <sup>h</sup>	Reactive intermediates <sup>h</sup>	Hypothetical: Alkylation of sulfhydryl groups <sup>i</sup> ; antineoplastic
Cadmium II chloride	10108-64-2	88	183.31	Organic compound; Cadmium compound	1400 mg/mL @ 20°C; ≥100 mg/mL @ 20°C	NA	NA	960	Kidney, liver toxin, corrosive	Yes <sup>j</sup>	No	Alters Ca <sup>++</sup> translocation, affects membrane ATPase & mitochondrial respiration.

**Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances**

Chemical	CASRN	LD <sub>50</sub> (mg/kg) <sup>a</sup>	MW (g/mol)	Chemical Class <sup>b</sup>	Water Solubility <sup>c</sup>	pK <sup>d</sup>	log Kow <sup>e</sup>	Boiling Point (°C) <sup>d</sup>	Toxic Effect Class <sup>f</sup>	Passage of Blood: Brain Barrier <sup>g</sup>	Metabolic Activation/ Inactivation <sup>f</sup>	Mechanism of Lethality <sup>f</sup>
Caffeine	58-08-2	192	194.20	Organic compound; Heterocyclic compound	21 mg/mL @ 25°C; 10-50 mg/mL @ 23°C	14 @ 25°C; pK <sub>b</sub> =14.15 @ 19°C	-0.07	17 (sublimes)	CNS stimulant	Free	No	Hypothetical: Inhibition of phosphodiesterase leading to AMP accumulation. Translocation of intracellular Ca <sup>++</sup> ? Adenosine receptor antagonism?; neurotoxic
Carbamazepine	298-46-4	1957	236.30	Organic compound; Heterocyclic compound	Practically insoluble	NA	2.45	NA	CNS depressant, hematotoxin	Free	10,11-epoxide metabolite as active as parent	Not known. Therapeutically decreases firing of noradrenergic neurons.
Carbon tetrachloride	56-23-5	2799	153.82	Organic compound; Halogenated hydrocarbon	0.793 mg/mL at 25°C; <1 mg/mL @ 21°C	NA	2.83	76.8	Liver, kidney toxin, CNS depressant	Free	More toxic intracellular metabolites?	Hypothetical: Covalent binding of toxic intracellular metabolites. Free radicals inducing lipid peroxidation?
Chloral hydrate	302-17-0	479	165.40	Organic compound; Alcohol	9310 mg/mL @ 25°C; ≥10 mg/mL @ 20.5°C	NA	0.99	96	CNS depressant & cardiotoxin	Freely	Active metabolite trichloroethanol is partly <sup>f</sup> or totally <sup>k</sup> responsible for CNS effect	Proposed: potentiation of GABA <sub>A</sub> receptor activity, inhibition of N-methyl-D-aspartate activity, & modulation of 5-hydroxytryptamine <sub>3</sub> receptor-mediated depolarization of the vagus nerve. <sup>k</sup>
Chloramphenicol	56-75-7	3393	323.14	Organic compound; Alcohol; Cyclic hydrocarbon; Nitro compound	2.5 mg/mL @ 25°C	NA	1.14	NA	Hematotoxin	Free	No	Hypothetical: Binds to mitochondrial ribosomes & inhibits enzyme syntheses (e.g., those necessary for oxidative phosphorylation)

**Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances**

Chemical	CASRN	LD <sub>50</sub> (mg/kg) <sup>a</sup>	MW (g/mol)	Chemical Class <sup>b</sup>	Water Solubility <sup>c</sup>	pK <sup>d</sup>	log Kow <sup>e</sup>	Boiling Point (°C) <sup>d</sup>	Toxic Effect Class <sup>f</sup>	Passage of Blood: Brain Barrier <sup>g</sup>	Metabolic Activation/ Inactivation <sup>f</sup>	Mechanism of Lethality <sup>f</sup>
Citric acid	77-92-9	3000	192.10	Organic compound; Carboxylic acid	592 mg/mL @ 20°C; ≥100 mg/mL @ 22°C	1=3.128 2=4.761 3=6.396 @ 25°C	-1.72	decomposes	Acidosis	NA	NA	NA
Colchicine	64-86-8	6 (mouse)	399.45	Organic compound; Polycyclic compound	45 mg/mL; ≥100 mg/mL @ 21°C	pK=12. 35 @ 20°C; pKa=1. 7 & 12.4	1.03		GI, liver, kidney, hemato-, PNS toxin	No	Not expected	Depresses respiratory center.
Cupric sulfate pentahydrate	7758-99-8	300	249.70	Inorganic compound; Sulfur compound; Metal	148 & 316 mg/mL @ 0°C; 2033 mg/mL @ 100°C; 230.5 mg/mL @ 25°C; 32 mg/mL @ 20°C; ≥100 mg/mL @ 21°C	NA	NA	decomposes @ 150°C	Liver, kidney toxin	Restricted	No	Hypothetical: Copper is reduced by thiol groups in cell membranes. superoxide is formed by reoxidation of copper, inducing lipid peroxidation.
Cycloheximide	66-81-9	2	281.40	Organic compound; Heterocyclic compound	21 mg/mL @ 2°C; 10-50 mg/mL @ 20°C	NA	0.55	NA	Liver toxin	Unknown	Metabolically activated	Inhibition of protein synthesis?; metabolic inhibitor
Dibutylphthalate	84-74-2	11998	278.30	Organic compound; Carboxylic acid	0.013 mg/mL @ 25°C; 0.01 mg/mL @ 20°C; <1 mg/mL @ 20°C	NA	4.9	340	CNS depressant; pulmonary, liver, testicular toxin	Yes <sup>p</sup>	Monobutyl metabolite has greater toxicity than parent in rats	Peroxisome proliferator <sup>q</sup>
Dichlorvos	62-73-7	17	220.98	Organic compound; Organophos- phorous compound	10 mg/mL @ 20°C; 5 g/mL; 10-50 mg/mL @ 20°C	NA	1.43, 1.45	245; 140 @ 20 mmHg	CNS depressant	Assumed due to CNS effects	Rapidly inactivated by hepatic metabolism	Inhibition of acetylcholinesterase resulting in acetylcholine accumulation in CNS & effector organs; irreversible cholinesterase inhibitor

**Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances**

Chemical	CASRN	LD <sub>50</sub> (mg/kg) <sup>a</sup>	MW (g/mol)	Chemical Class <sup>b</sup>	Water Solubility <sup>c</sup>	pK <sup>d</sup>	log Kow <sup>e</sup>	Boiling Point (°C) <sup>d</sup>	Toxic Effect Class <sup>f</sup>	Passage of Blood: Brain Barrier <sup>g</sup>	Metabolic Activation/ Inactivation <sup>f</sup>	Mechanism of Lethality <sup>f</sup>
Diethyl phthalate	84-66-2	8602	222.20	Organic compound; Carboxylic acid	<1 mg/mL @ 19 °C and 25 °C	NA	2.47	298	CNS depressant, liver toxin	Yes <sup>m</sup>	Monoethyl metabolite has greater toxicity than parent in rats	Peroxisome proliferator <sup>n</sup>
Digoxin	20830-75-5	18 (mouse)	780.90	Organic compound; Polycyclic compound; Carbohydrate	0.0648 mg/mL @ 25 °C	NA	1.26	NA	Cardiotoxin	Restricted	Also active metabolites	Impairs ion transport & increases sarcoplasmic calcium by binding to Na <sup>+</sup> /K <sup>+</sup> ATPase, increasing automaticity of cardiac cells.
Dimethylformamide	68-12-2	2800	73.10	Organic compound; Amide	Miscible; ≥100 mg/mL @ 22 °C	-0.01 @ -20 °C	-1.01	153	Liver, kidney toxin	NA	NA	Hepatocellular necrosis <sup>n</sup>
Diquat dibromide monohydrate	6385-62-2	231	362.10	Organic compound; Heterocyclic compound	700 mg/mL @ 20 °C; ≥100 mg/mL @ 20 °C	NA	-3.05	NA	GI, pulmonary, liver, kidney toxin	Free <sup>n</sup>	No <sup>n</sup>	Assumed to be same as Paraquat; Hypothetical: Multisystem failure due to depletion of superoxide dismutase, formation of free radicals & lipid peroxidation. Lung fibrosis due to accumulation.
Disulfoton	298-04-4	2	274.42	Organic compound; Organo- phosphorous compound; Sulfur compound	0.012 mg/mL @ 20 °C	NA	4.02	132-33 @ 1.5 mmHg; 108 and 62 @ 0.01 mmHg	CNS depressant	Yes	More toxic metabolites	Inhibition of acetylcholinesterase resulting in acetylcholine accumulation in CNS & effector organs; irreversible cholinesterase inhibitor
Endosulfan	115-29-7	18	406.91	Organic compound; Heterocyclic compound; Sulfur compound	0.00053 mg/mL @ 25 °C	NA	3.83	106 @ 0.7 mm, partial decom- position	CNS depressant	Yes <sup>o</sup>	No <sup>o</sup>	Affects brain neurotransmitter levels. <sup>o</sup>

**Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances**

Chemical	CASRN	LD <sub>50</sub> (mg/kg) <sup>a</sup>	MW (g/mol)	Chemical Class <sup>b</sup>	Water Solubility <sup>c</sup>	pK <sup>d</sup>	log Kow <sup>e</sup>	Boiling Point (°C) <sup>d</sup>	Toxic Effect Class <sup>f</sup>	Passage of Blood: Brain Barrier <sup>g</sup>	Metabolic Activation/Inactivation <sup>f</sup>	Mechanism of Lethality <sup>f</sup>
Epinephrine bitartrate	51-42-3	4 (mouse)	333.30	Organic compound; Alcohol; Amine	1 mg/mL @ 25°C; < 0.1 mg/mL @ 18°C (for base)	NA	-1.52	NA	Cardiovascular toxin	No	Large first pass metabolism to inactive metabolites	Adrenergic receptor stimulation.
Ethanol	64-17-5	14008	46.07	Organic compound; Alcohol	>10% why include; ≥ 100 mg/ml @ 23°C	15.9 @ 25°C	-0.31	78.5	CNS depressant	Free	Acetaldehyde, active metabolite	Hypothetical: Interferes with cell membrane fluidity, perturbing proteins such as ion channels. Depression of postsynaptic potentials in CNS; solvent
Ethylene glycol	107-21-1	8567	62.07	Organic compound; Alcohol	Miscible; ≥ 100 mg/mL @ 17.5°C	NA	-1.36	197.6 @ 760 mmHg	CNS depressant, kidney toxin	Free	Glyoxalate, glycolate, & oxalate, active metabolites	Hypothetical: Metabolites inhibit mitochondria to produce metabolic acidosis. Oxalate decreases sarcoplasmic Ca <sup>++</sup> ; affects kidney function; oxalic acid is toxic metabolite
Fenpropathrin	39515-41-8	18	349.43	Organic compound; Nitrile; Ester; Ether	0.00033 mg/mL @ 25°C	NA	6.0 @ 20°C	377	PNS toxin	Yes <sup>p</sup>	Rapidly hydrolyzed to inactive products in mammals <sup>e,p</sup>	Delays closure of sodium channel causing persistent depolarization of membrane.
Gibberellic acid	77-06-5	6305	346.38	Organic compound; Polycyclic compound	5 mg/mL; slightly	4	0.24	NA	NA	NA	NA	NA
Glutethimide	77-21-4	600	217.30	Organic compound; Heterocyclic compound	Practically insoluble	4.2	1.9	NA	CNS depressant	Presumed	2X active metabolite: 4-hydroxyglutethimide	CNS depression; anticholinergic activity
Glycerol	56-81-5	12691	92.09	Organic compound; Alcohol	Soluble in all proportions; ≥ 100 mg/mL @ 18°C	14.4	-1.76	182; 290 @ 760 mmHg, decomposes	Body fluids	No evidence found	No	Cellular dehydration; osmotic effect

**Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances**

Chemical	CASRN	LD <sub>50</sub> (mg/kg) <sup>a</sup>	MW (g/mol)	Chemical Class <sup>b</sup>	Water Solubility <sup>c</sup>	pK <sup>d</sup>	log Kow <sup>e</sup>	Boiling Point (°C) <sup>d</sup>	Toxic Effect Class <sup>f</sup>	Passage of Blood: Brain Barrier <sup>g</sup>	Metabolic Activation/Inactivation <sup>f</sup>	Mechanism of Lethality <sup>f</sup>
Haloperidol	52-86-8	128	375.90	Organic compound; Ketone	0.014 mg/mL	8.3	3.36	NA	CNS depressant	Presumed	No	Blocks dopamine receptors
Hexachlorophene	70-30-4	61	406.91	Organic compound; Cyclic hydrocarbon; Phenol	0.140 mg/mL @ 25 °C; < 1 mg/mL @ 20 °C	4.95	6.91	NA	CNS depressant	Restricted	No	Hypothetical: Uncoupling of oxidative phosphorylation. Binding to proteins in cytoplasmic membrane & cell organelles.
Lactic acid	50-21-5	3730	90.08	Organic compound; Carboxylic acid	Soluble	3.86 @ 25 °C	-0.72	122 @ 14-15 mmHg	Acidosis, corrosive	Yes <sup>g</sup>	Unknown	Disturbance of metabolism (lactic acidosis).
Lindane	58-89-9	76	290.80	Organic compound; Halogenated hydrocarbon	0.0073 mg/mL @ 25 °C; < 1 mg/mL @ 24 °C	NA	3.72	323.4 @ 760 mmHg	CNS stimulant	Free	No?	CNS depression through inhibition of GABA receptor linked chloride channel at the picrotoxin binding site, leading to blockade of chloride influx into neurons?
Lithium I carbonate	554-13-2	1187 (sulfate salt; mouse)	73.89	Inorganic compound; Lithium compound; Alkalies; Inorganic carbon compound	1.5 mg/mL @ 0 °C; 1.3 mg/mL @ 20 °C; 1.2 mg/mL @ 40 °C; 12.2 mg/mL cold; 7 mg/mL hot	NA	NA	NA	CNS depressant	Restricted (assumed same as lithium sulfate)	No	Unknown: Partial substitution for normal cations of cells may disturb energy processes?
Meprobamate	57-53-4	794	218.30	Organic compound; Carboxylic acid	3.4 mg/mL @ 20 °C; 7.9 mg/mL @ 37 °C; < 1 mg/mL @ 20 °C	9.2	NA	NA	CNS depressant cardiotoxin	NA	Rapidly inactivated by hepatic metabolism	Unknown

**Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances**

Chemical	CASRN	LD <sub>50</sub> (mg/kg) <sup>a</sup>	MW (g/mol)	Chemical Class <sup>b</sup>	Water Solubility <sup>c</sup>	pK <sup>d</sup>	log Kow <sup>e</sup>	Boiling Point (°C) <sup>d</sup>	Toxic Effect Class <sup>f</sup>	Passage of Blood: Brain Barrier <sup>g</sup>	Metabolic Activation/ Inactivation <sup>f</sup>	Mechanism of Lethality <sup>f</sup>
Mercury II chloride	7487-94-7	1	271.50	Inorganic compound; Mercury compound; Chlorine compound	69 mg/mL at 20°C; 5-50 mg/mL @ 22°C	NA	0.22	302	Corrosive, kidney toxin	Restricted	No	Hypothetical: Changes membrane potentials & blocks enzyme reactions in cells by targeting the sulfhydryl part of active sites of some enzymes.
Methanol	67-56-1	13012	32.04	Organic compound; Alcohol	Completely miscible at 20°C; ≥100 mg/mL @ 21°C	15.3	-0.77	64.7 @ 760 mmHg	CNS depressant	Free	Active metabolites: formaldehyde, formic acid	Hypothetical: Accumulation of formic acid leads to metabolic acidosis. Lactate inhibits mitochondrial respiration; formaldehyde metabolite
Nicotine	54-11-5	50	162.20	Organic compound; Heterocyclic compound	Miscible below 60°C	pK <sub>b1</sub> =6. 16 @ 15°C; pK <sub>b2</sub> =1 0.96	1.17	247	CNS stimulant	Free	No	CNS nicotinic receptor; cholinergic block causing polarization of CNS and PNS synapses.
Paraquat	1910-42-5	58	257.20	Organic compound; Heterocyclic compound	Soluble; ≥100 mg/mL @ 19°C	NA	-4.22 @ pH 7.4	175-180 @ 760 mmHg, decomposes	Pulmonary toxin	Free?	No	Multisystem failure due to depletion of superoxide dismutase, with formation of free radicals & lipid peroxidation. Lung fibrosis due to accumulation; interferes with ATP synthesis.
Parathion	56-38-2	2	291.28	Organic compound; Organo- phosphorous compound; Sulfur compound	0.011 mg/mL @ 20°C; <1 mg/mL @ 23°C	NA	3.83	375 @ 760 mm Hg	CNS depressant	Free (assumed the same as malathion)	Paraoxon is active metabolite.	Inhibition of acetylcholinesterase resulting in acetylcholine accumulation in CNS & effector organs; irreversible cholinesterase inhibitor

**Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances**

Chemical	CASRN	LD <sub>50</sub> (mg/kg) <sup>a</sup>	MW (g/mol)	Chemical Class <sup>b</sup>	Water Solubility <sup>c</sup>	pK <sup>d</sup>	log Kow <sup>e</sup>	Boiling Point (°C) <sup>d</sup>	Toxic Effect Class <sup>f</sup>	Passage of Blood: Brain Barrier <sup>g</sup>	Metabolic Activation/Inactivation <sup>f</sup>	Mechanism of Lethality <sup>f</sup>
Phenobarbital	50-06-6	163	232.23	Organic compound; Heterocyclic compound	1 mg/mL; 1.3 mg/mL at 25°C; <0.1 mg/mL @ 14°C	pK <sub>1</sub> =7.3, pK <sub>2</sub> =11.8	1.47	NA	CNS depressant	Free	No	Neurotoxic; CNS depression through inhibition of GABA synapses? Inhibits hepatic NADH cytochrome oxidoreductase;
Phenol	108-95-2	414	94.11	Organic compound; Phenol	67 mg/mL; 82.8 mg/mL @ 25°C; 93 mg/mL @ 25°C; 50-100 mg/mL @ 19°C	NA	1.46	182 @ 760 mm Hg	Corrosive; CNS depressant	Free	No	General protoplasmic poison that denatures proteins; depresses vasomotor center
Phenylthiourea	103-85-5	3.0	152.20	Organic compound; Sulfur compound; Urea	2.5 mg/mL @ 25°C; <1 mg/mL @ 21°C	NA	0.71	NA	Pulmonary toxin	NA	Humans & animals have high capacity to detoxify sulfides	Destroys cytochrome p450; interferes with pulmonary, thyroid functions.
Physostigmine	57-47-6	4.5	275.40	Organic compound; Carboxylic acid; Heterocyclic compound	Slightly soluble	NA	NA	NA	CNS depressant	Easily	None known	Inhibition of acetylcholinesterase resulting in acetylcholine accumulation in CNS & effector organs.
Potassium I chloride	7447-40-7	2602	74.55	Inorganic compound; Potassium compound; Chlorine compound	342 mg/mL @ 20°C; >100 mg/mL @ 20°C	NA	NA	1500	Cardiotoxin	Free?	No	Essential cellular electrolyte maintains normal transmembrane potential, necessary for heart conduction.
Potassium cyanide	151-50-8	10	65.12	Inorganic compound; Potassium compound; Nitrogen compound	500 mg/mL cold; 1000 mg/mL hot	NA	NA	NA	CNS stimulant, corrosive	Free	No	General enzyme inhibition. Interferes with ATP synthesis. High affinity for Fe <sup>+++</sup> . Inhibits cell respiration by inhibition of cytochrome oxidase.
Procainamide HCl	51-06-9	1950	271.79	Organic compound; Carboxylic	Freely soluble	NA	NA	NA	CNS depressant, cardiotoxin	Some	Less potent <sup>f</sup> ; active metabolite <sup>e</sup>	Slows impulse conduction in the heart? <sup>f</sup>

**Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances**

Chemical	CASRN	LD <sub>50</sub> (mg/kg) <sup>a</sup>	MW (g/mol)	Chemical Class <sup>b</sup>	Water Solubility <sup>c</sup>	pK <sup>d</sup>	log Kow <sup>e</sup>	Boiling Point (°C) <sup>d</sup>	Toxic Effect Class <sup>f</sup>	Passage of Blood: Brain Barrier <sup>g</sup>	Metabolic Activation/Inactivation <sup>f</sup>	Mechanism of Lethality <sup>f</sup>
				acid; Amide								
2-Propanol	67-63-0	5843	60.10	Organic compound; Alcohol	≥100 mg/mL @ 22°C	NA	0.05	82.3	CNS depressant	Free	No.	CNS depression through membrane effects <sup>h</sup>
Propranolol HCl	350-60-90	470 (mouse)	295.80	Organic compound; Alcohol; Amine; Polycyclic compound	Soluble	NA	3.09	NA	Cardiotoxin	Free	No?	Unknown: Beta-adrenergic blockade?
Propylparaben	94-13-3	6326 (mouse)	180.20	Organic compound; Carboxylic acid; Phenol	0.463 mg/mL @ 25°C; <1 mg/mL @ 12°C	NA	3.04	NA	CNS depressant	NA	NA	NA
Sodium arsenite	7784-46-5	41	129.90	Inorganic compound; Arsenical; Sodium compound	Very to freely soluble	NA	NA	NA	PNS, liver, hematotoxin	Yes	Not expected	Assumed the same as arsenic trioxide - causes multisystem failure due to uncoupling of oxidative phosphorylation & inhibition of pyruvate & succinate oxidative pathways.
Sodium chloride	7647-14-5	2998	58.44	Inorganic compound; Sodium compound; Chlorine compound	357 mg/mL @ 0°C; 391.2 mg/mL @ 100°C	NA	NA	1413°C	Body fluids	Restricted	No	Acute dehydration of brain cells caused by osmotic shift of water to the outside of the blood:brain barrier.
Sodium dichromate dihydrate	7789-12-0	50	298.00	Inorganic compound; Sodium compound; Chromium compound	2380 mg/mL @ 0°C	NA	NA	decomposes @ 400	Kidney, liver toxin	Yes <sup>s</sup>	Less active in presence of metabolizing system	Inhibition of respiratory chain activity; carcinogenic system

**Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances**

Chemical	CASRN	LD <sub>50</sub> (mg/kg) <sup>a</sup>	MW (g/mol)	Chemical Class <sup>b</sup>	Water Solubility <sup>c</sup>	pK <sup>d</sup>	log Kow <sup>e</sup>	Boiling Point (°C) <sup>d</sup>	Toxic Effect Class <sup>f</sup>	Passage of Blood: Brain Barrier <sup>g</sup>	Metabolic Activation/Inactivation <sup>f</sup>	Mechanism of Lethality <sup>f</sup>
Sodium I fluoride	7681-49-4	180	41.99	Inorganic compound; Sodium compound; fluorine compound	43 mg/mL @ 25°C; 10-50 mg/mL @ 23°C	NA	NA	NA	GI irritant, CNS depressant	Restricted	No	Hypothetical: Protoplasmic poison interfering with many enzymes. May lower sarcoplasmic Ca <sup>++</sup> & induce K <sup>+</sup> efflux from cells.
Sodium hypochlorite	7681-52-9	8910	74.44	Inorganic compound; Sodium compound; Oxygen compound; chlorine compound	293 mg/mL @ 0°C	NA	NA	111	Corrosive, body fluids	NA	NA	NA
Sodium oxalate	62-76-0	155	134.00	Organic compound; Carboxylic acid	220 mg/mL @ 25°C	NA	NA	NA	Corrosive, body fluids, kidney & cardiotoxin, CNS depressant	Restricted	No	Hypothetical: Ca <sup>++</sup> -complexing action, depressing the level of ionized Ca <sup>++</sup> in body fluids, but doesn't explain action on GI, vasculature, & kidney. Corrosivity not due to acidity.
Sodium selenate	13413-01-0	1.6	188.90	Inorganic compound; Sodium compound; Selenium compound	≥ 100 mg/mL @ 21°C	NA	NA	NA	Liver, kidney toxin	Yes <sup>t</sup>	Not expected	Inactivates sulfhydryl enzymes for oxidative reactions in cellular respiration. <sup>t</sup>
Strychnine	57-24-9	2	334.40	Organic compound; Heterocyclic compound	0.16 mg/mL @ 25°C	8.26 @ 25°C	1.93	270 @ 5 mmHg	CNS stimulant	Expected	No	Increases glutamic acid in the CNS. Alkaloid poison.

**Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances**

Chemical	CASRN	LD <sub>50</sub> (mg/kg) <sup>a</sup>	MW (g/mol)	Chemical Class <sup>b</sup>	Water Solubility <sup>c</sup>	pK <sup>d</sup>	log Kow <sup>e</sup>	Boiling Point (°C) <sup>d</sup>	Toxic Effect Class <sup>f</sup>	Passage of Blood: Brain Barrier <sup>g</sup>	Metabolic Activation/ Inactivation <sup>f</sup>	Mechanism of Lethality <sup>f</sup>
Thallium I sulfate	7446-18-6	29 (mouse)	504.80	Inorganic compound; Metal; Sulfur compound	48.7 mg/mL @ 15°C; 191.4 mg/mL @ 100°C	NA	NA	NA	GI irritant, CNS toxin (encephalo- pathy)	Restricted	No	Hypothetical: Enzyme inhibition by binding sulfhydryl groups of mitochondrial membranes. Interferes with oxidative phosphorylation by inhibition of Na <sup>+</sup> /K <sup>+</sup> ATPase.
Trichloroacetic acid	76-03-9	4999	163.40	Organic compound; Carboxylic acid	10 g/mL @ 25°C; 1200 mg/mL @ 25°C; 13.06 g/mL @ 25°C; ≥100 mg/mL @ 22°C	NA	1.33	196	GI corrosion, acidosis	Expected	Not expected	Corrosive; possible carcinogen
1,1,1-Trichloroethane	71-55-6	10298	133.41	Organic compound; Halogenated hydrocarbon	4.4 mg/mL @ 20°C; <1 mg/mL @ 20°C	NA	2.49	76	CNS depressant; liver toxin	Free	No.	Arrhythmogenic <sup>u</sup>
Triethylenemelamine	51-18-3	1.0	204.23	Organic compound; Heterocyclic compound	400 mg/mL @ 26°C; <1 mg/mL @ 16°C	NA	-0.54	139 (decomposes)	Hemato-, liver, kidney toxin	Unknown	Expected since it's an alkylator	Genotoxic; binds with DNA; alkylating agent; alkylates proteins
Triphenyltin hydroxide	76-87-9	44	367.02	Organic compound; Organo- metallic compound	0.0012 mg/mL; <1 mg/mL @ 21°C	NA	NA	NA	CNS toxin (encephalo- pathy), skin & GI irritant	Rapidly	No	Affects a number of enzymes involved in cellular energy production and use. Affects immune system; causes lymphopenia; clastogenic
Valproic acid	99-66-1	670 (mouse)	144.20	Organic compound; Carboxylic acid; Lipids	2 mg/mL @ 20°C; 1.27 mg/mL; <1 mg/mL @ 22°C	NA	2.75	220	CNS depressant, liver toxin	Yes	Some metabolites may be active	Increases GABA in the CNS?

**Table F-2 Chemical, Physical, and Biological Information from the Literature for the 72 Reference Substances**

Chemical	CASRN	LD <sub>50</sub> (mg/kg) <sup>a</sup>	MW (g/mol)	Chemical Class <sup>b</sup>	Water Solubility <sup>c</sup>	pK <sup>d</sup>	log Kow <sup>e</sup>	Boiling Point (°C) <sup>d</sup>	Toxic Effect Class <sup>f</sup>	Passage of Blood: Brain Barrier <sup>g</sup>	Metabolic Activation/ Inactivation <sup>f</sup>	Mechanism of Lethality <sup>f</sup>
Verapamil HCl	152-11-4	108	491.08	Organic compound; Amine	70 mg/mL	NA	3.79	NA	Cardiotoxin	Restricted?	Also active metabolites	Inhibition of transmembrane Ca <sup>++</sup> flux in excitatory tissues. Cardiac-Ca <sup>++</sup> channel blocker. Also alpha-adrenergic blockade.
Xylene	1330-20-7	4300	106.17	Organic compound; Cyclic hydrocarbon	Practically insoluble; <1 mg/mL @ 22°C	NA	3.12-3.2	136-140	CNS depressant	Free	No	Unknown: Heart failure caused by sensitization of heart to catecholamines?; solvent

Abbreviations: MW=Molecular weight; NA=No information found; NADPQI=N-acetyl-*p*-benzoquinoneimine; CNS=Central nervous system; AMP=Adenosine monophosphate; GABA=Gamma aminobutyric acid; GI=Gastrointestinal; PNS=Peripheral nervous system; NADH=Nicotine adenine dinucleotide (reduced).

<sup>a</sup>LD<sub>50</sub> data from Registry of Cytotoxicity (Halle 1998), Hazardous Substances Data Bank (NLM 2001, 2002), or Registry of Toxic Effects of Chemical Substances® (MDL information Systems 2001, 2002). Rat data unless otherwise noted. Rounded to the nearest one.

<sup>b</sup>Based on the Medical Subject Heading [MeSH] index (NLM 2005).

<sup>c</sup>Hazardous Substances Data Bank (NLM 2001, 2002) and NTP Chemical Health and Safety Data (2001) at [http://ntp-server.niehs.nih.gov/Main\\_Pages/Chem-HS.html](http://ntp-server.niehs.nih.gov/Main_Pages/Chem-HS.html). The NTP database is no longer available. NTP values can be identified by the use of the following symbols: <, >, and ≥. Conditions are reported if available.

<sup>d</sup>Hazardous Substances Data Bank (NLM 2001, 2002) unless otherwise specified. pK measured under the conditions specified. If no conditions were specified, none are reported.

<sup>e</sup>Hazardous Substances Data Bank (NLM 2001, 2002) or Material Safety Data Sheets. Boiling point measured under the conditions specified. If no conditions were specified, none are given.

<sup>f</sup>Ekwall et al. (1998) or Hazardous Substances Data Bank (NLM 2001, 2002) unless otherwise noted.

<sup>g</sup>Cosmetic Ingredient Review Panel (1983).

<sup>h</sup>Orphan Medical (1999).

<sup>i</sup>Glaxo Wellcome (2000).

<sup>j</sup>ATSDR (1999).

<sup>k</sup>EPA (2000b).

<sup>l</sup>ATSDR (2001).

<sup>m</sup>ATSDR (1995).

<sup>n</sup>EPA (1995).

<sup>o</sup>ATSDR (2000a).

<sup>p</sup>ATSDR (2004a).

<sup>q</sup>Ames (2000).

<sup>r</sup>Hardman et al. (1996).

<sup>s</sup>ATSDR (2000b).

<sup>t</sup>ATSDR (2004b).

<sup>u</sup>Casarett et al. (2001).

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## **Appendix F3**

### **Candidate Reference Substances**

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### F.3 Candidate Reference Substances

#### F.3.1 Sources of Candidate Substances

The process of identifying the 72 reference substances started with the compilation of a database that ultimately contained 116 candidate substances. The intent of the SMT was to compile a database with more than 12 substances in each toxicity category that also met the other criteria, and then to prioritize the substances in each category to select the 72 reference substances to be tested. As recommended by the Workshop 2000 participants (ICCVAM 2001a), the following publicly available databases and other indicated sources were used to identify candidate chemicals:

- The MEIC program, which collected human toxicity data and *in vitro* toxicity data from 61 test methods for the first 50 chemicals (Ekwall et al. 1998). The ECVAM members of the SMT preferred these chemicals since human acute toxicity data had already been collected.
- The RC (Halle 1998, 2003), which contains a compilation of *in vitro* cytotoxicity and *in vivo* rodent LD<sub>50</sub> data for 347 chemicals
- The Toxic Exposure Surveillance System (TESS) (Litovitz et al. 2000), which compiles reports of toxic human exposures from poison control centers throughout the United States
- Pesticides recommended for consideration by the EPA Office of Pesticide Programs (OPP)
- The *Guidance Document* (ICCVAM 2001b), which reported *in vitro* NRU results for 11 RC chemicals using protocols similar to those used in the NICEATM/ECVAM validation study
- The U.S. NTP test database, which contains information on the toxicity of chemicals relevant to human exposure (NTP 2002)
- The EPA High Production Volume (HPV) Challenge Program, which is a voluntary testing program to provide the public with a complete set of baseline health and environmental effects data for each chemical that is manufactured within or imported into the United States at amounts > 1 million pounds/year (EPA 2000a)

### F.3.2 Selection of Candidate Substances

The 116 candidate substances consisted of the 72 reference substances selected for testing in the NICEATM/ECVAM validation study (see **Table 3-2**) and the alternate substances that were not selected for testing (see **Table F3-1**). The alternate candidate substances in **Table F3-1** are grouped by GHS acute oral toxicity classification. For each reference substance, the table provides the corresponding rat or mouse oral LD<sub>50</sub> value, the database(s) or other source(s) used to identify the chemical as a potential candidate, notes on volatility and/or DEA restrictions, and the type of product and/or use for the substance. Product/use categories were identified from HSDB (NLM 2001, 2002) or RTECS<sup>®</sup> (MDL Information Systems 2001, 2002).

The final list of candidate substances, which includes the substances in **Table 3-2** and **Table F3-1**, included:

- Sixty-five MEIC chemicals. These include the first 50 chemicals evaluated by MEIC as well as another 15 chemicals that were identified for future evaluation (C. Clemedson, personal communication 2001). Twenty of these chemicals were identified for the EDIT program, a follow-on project to the MEIC study to develop supplementary toxicity and kinetic tests (to determine distribution of chemicals in the body and biotransformation of chemicals to more toxic metabolites) to improve the prediction of human toxicity by the battery of tests identified as the best predictors in the MEIC program (Clemedson et al. 2002). The EDIT chemicals were selected by excluding MEIC chemicals that were volatile, those that precipitated at the IC<sub>50</sub> dose level, and those with sparse or insufficient data on human toxicity or mechanism of acute toxicity.
- Sixteen pesticides with extensive human exposure nominated by the EPA OPP. These included fenpropathrin, endosulfan, bromoxynil (phenol), fipronil, carbaryl, rotenone, metaldehyde, molinate, 1,3-dichloropropene, dichlorvos, chlorpyrifos, sodium arsenite, triphenyltin hydroxide,

cycloheximide, acrolein, and boric acid. Pentachlorophenol was also nominated, but was already on the candidate list since it was a MEIC chemical.

- Five substances associated with the highest incidence of toxic exposures reported by U.S. poison control centers participating in the TESS (Litovitz et al. 2000): hypochlorite, acetaminophen, ethanol, diphenhydramine, and isopropanol. The five chemicals with the greatest incidence of toxic exposures among children were the same, except that oxalate replaced ethanol. Most of these chemicals were already identified as candidate substances due to their inclusion in the MEIC study. Since hypochlorite (sodium salt) and diphenhydramine, were not already included, they were added to the list of candidates.
- Eleven substances recommended in the *Guidance Document* (ICCVAM 2001b) for qualifying *in vitro* cytotoxicity assays for the prediction of starting doses using the RC regression. These substances were recommended because the IC<sub>50</sub> and LD<sub>50</sub> data for these substances fit the RC regression line extremely well. These chemicals were sodium dichromate dihydrate, cadmium chloride, p-phenylenediamine, DL-propranolol HCl, trichlorfon, ibuprofen, nalidixic acid, salicylic acid, antipyrine, dimethylformamide, and glycerol
- Sixteen substances from the NTP database
  - Furfural, methyleugenol, and methylphenidate, scheduled for testing by the NTP National Center for Toxicogenomics (NCT) (G. Boorman, personal communication 2001), were added. Acetaminophen, another hepatotoxin to be tested by the NCT, was already a candidate substance because it was included in the MEIC study. Chromium (VI), recommended by the NTP for consideration due to the potential for human exposure via drinking water (NTP 2002) was represented in the list of candidate substances by sodium dichromate dihydrate, which was also recommended in the *Guidance Document* (ICCVAM 2001b).

- Dibutyl phthalate, 5-aminosalicylic acid, propylparaben, gibberellic acid, and diethyl phthalate were added to increase the number of chemicals with  $LD_{50}$  values  $>5000$  mg/kg.
- Trichloroacetic acid was added to increase the number of substances in the  $2000 < LD_{50} \leq 5000$  mg/kg category.
- Sodium selenate was added to increase the number of chemicals in the  $LD_{50} \leq 5$  mg/kg category to 12.
- Six chemicals that were also on the HPV list were added. Lactic acid, citric acid, and acetonitrile were added to increase the number of chemicals in the  $2000 < LD_{50} \leq 5000$  mg/kg category. Tert-butylamine, 2,4-dinitrophenol, and acrolein were added to increase the number of chemicals in the  $5 < LD_{50} \leq 50$  mg/kg category.
- Eight additional RC substances in the  $LD_{50} \leq 5$  mg/kg category. These were: triethylenemelamine, busulfan, disulfoton, parathion, aminopterin, phenylthiourea, epinephrine bitartrate, and aflatoxin B1.

The goal to identify more than 12 candidate substances for each toxicity category was unrealized for three toxicity categories. The most toxic category ( $LD_{50} \leq 5$  mg/kg), and least toxic categories ( $2000 < LD_{50} \leq 5000$  mg/kg,  $LD_{50} > 5000$  mg/kg), contained only 12 candidate substances each. The intermediate toxicity categories ( $50 < LD_{50} \leq 300$  mg/kg,  $> 300 < LD_{50} \leq 2000$  mg/kg), however, contained two to three times the minimum number of candidate chemicals.

**Table F3-1 Alternate Candidate Substances**

GHS Category <sup>1</sup> /Chemical	Rodent Oral LD <sub>50</sub> <sup>2</sup> (mg/kg)	Source <sup>3</sup>	Notes <sup>5</sup>	Product/Use <sup>4</sup>
<i>LD<sub>50</sub> ≤ 5 mg/kg</i>				
Aflatoxin B1	5.0	RC (outlier)	Prohibitively expensive	Food contaminant
<i>5 &lt; LD<sub>50</sub> ≤ 50 mg/kg</i>				
2,4-Dinitrophenol	30	RC (outlier), NTP, HPV		Pesticide (fungicide/ insecticide) manufacturing
t-Butylamine	44 <sup>a</sup>	EPA, NTP, HPV		Manufacturing
Acrolein	46	RC, TESS, EPA, NTP, HPV	Volatile (BP=52°C)	Pesticide (herbicide/ rodenticide/ algicide), manufacturing
<i>50 &lt; LD<sub>50</sub> ≤ 300 mg/kg</i>				
Pentachlorophenol	51	MEIC, RC (outlier), NTP		Disinfectant
Amphetamine sulfate	55	MEIC, EDIT, RC (outlier), TESS, NTP	DEA	Pharmaceutical (stimulant)
Rotenone	60	RC, TESS, EPA, NTP		Pesticide (insecticide/ piscicide)
Furfural	65 <sup>a</sup>	NTP, HPV		Solvent, food additive
p-Phenylenediamine	80	RC, GD, NTP, HPV		Dyeing
Chlorpyrifos	82 <sup>a</sup>	TESS, EPA, NTP		Pesticide (insecticide)
Dextropropoxyphene HCl	83	MEIC, RC (outlier), TESS		Pharmaceutical (analgesic)
Methadone	86 <sup>a</sup>	MEIC, TESS, NTP	DEA	Pharmaceutical (analgesic)
Fipronil	92 <sup>a</sup>	EPA		Pesticide (insecticide)
Pentobarbital	125	MEIC, RC TESS	DEA	Pharmaceutical (sedative)
Bromoxynil (phenol)	190 <sup>a</sup>	EPA		Pesticide (herbicide)
Diphenylhydantoin	199	MEIC, RC, TESS, NTP		Pharmaceutical (anticonvulsant)
Metaldehyde	227 <sup>a</sup>	TESS, EPA		Pesticide (molluscicide)
Carbaryl	230	RC, EPA, NTP		Pesticide (insecticide)
<i>300 &lt; LD<sub>50</sub> ≤ 2000 mg/kg</i>				
Ferrous sulfate	319	MEIC, RC, TESS		Food additive
Warfarin	324	MEIC, RC, TESS, EPA		Pharmaceutical (anticoagulant), pesticide
Disopyramide	333 <sup>a</sup>	MEIC, TESS		Pharmaceutical (antiarrhythmic)
Barium II nitrate	355	MEIC, RC, TESS, NTP		Pyrotechnic
Thioridazine HCl	358	MEIC, RC, TESS		Pharmaceutical (antipsychotic)
Methylphenidate	367 <sup>a</sup>	NTP	DEA	Pharmaceutical (stimulant)
Molinate	369 <sup>a</sup>	EPA, NTP		Pesticide (herbicide)

**Table F3-1 Alternate Candidate Substances**

GHS Category <sup>1</sup> /Chemical	Rodent Oral LD <sub>50</sub> <sup>2</sup> (mg/kg)	Source <sup>3</sup>	Notes <sup>5</sup>	Product/Use <sup>4</sup>
2,4-Dichlorophenoxy-acetic acid	369	MEIC, RC, TESS, EPA, NTP, HPV		Pesticide (herbicide)
Orphenadrine HCl	425	MEIC, RC, NTP		Pharmaceutical (analgesic)
Trichlorfon	451	RC, EPA, GD, NTP		Pesticide (insecticide)
Quinidine sulfate	456	MEIC, RC, NTP (base)		Pharmaceutical (antiarrhythmic)
1,3-Dichloropropene	470 <sup>a</sup>	TESS, EPA, NTP		Pesticide (nematocide)
Theophylline	600 <sup>b</sup>	MEIC, RC, TESS, NTP		Pharmaceutical (antiasthmatic)
Isoniazid	650	MEIC, RC, TESS, NTP		Pharmaceutical (antibiotic)
Diazepam	709	MEIC, EDIT, RC, TESS, NTP	DEA	Pharmaceutical (anxiolytic)
Maprotiline	760 <sup>a</sup>	MEIC, TESS		Pharmaceutical (antidepressant)
Methyleugenol	810 <sup>a</sup>	NTP		Food additive
Diphenhydramine HCl	855	MEIC, RC, TESS, NTP		Pharmaceutical (antihistamine)
Malathion	885	MEIC, EDIT, RC, TESS, EPA, NTP		Pesticide (insecticide)
Salicylic acid	891	RC, TESS, GD, NTP, HPV		Pharmaceutical (analgesic)
Chloroform	908	MEIC, RC, NTP, HPV	Volatile (BP=61°C)	Solvent
Chloroquine diphosphate	970	MEIC, RC		Pharmaceutical (antimalarial)
Ibuprofen	1009	RC, TESS, GD		Pharmaceutical (analgesic)
Nalidixic acid	1349	RC, GD, NTP		Pharmaceutical (antibiotic)
Dichloromethane	1597	MEIC, RC, TESS, NTP, HPV	Volatile (BP=40°C)	Solvent
Antipyrine	1800	RC, GD		Pharmaceutical (analgesic)

<sup>1</sup>GHS=Globally Harmonized System of Classification and Labelling of Chemicals for acute oral toxicity (UN 2005).

<sup>2</sup>LD<sub>50</sub> data are from the Registry of Cytotoxicity (Halle 1998) and are for rats, the preferred species for oral acute toxicity studies, unless otherwise noted. Data with decimal places are rounded to the nearest one.

<sup>3</sup>Sources used to identify candidate chemicals: EDIT=Evaluation-guided Development of New *In Vitro* Test Batteries; EPA=Pesticides registered with the Environmental Protection Agency; EHS=EPA's Extremely Hazardous Substance list; HPV=High Production Volume chemicals (i.e., those that are imported into or produced in the United States in amounts ≥ 1,000,000 lbs/year; GD=*Guidance Document* (ICCVAM 2001b); MEIC=Multicentre Evaluation of *In Vitro* Cytotoxicity; NTP=National Toxicology Program; RC=Registry of Cytotoxicity with chemicals classified as regression outliers shown in parentheses; TESS=Toxic Exposure Surveillance System (Litovitz et al. 2000).

<sup>4</sup>Product/use categories from Hazardous Substances Data Bank (NLM 2002) or Registry of Toxic Effects of Chemical Substances ([RTECS<sup>®</sup>], MDL Information Systems 2002). Pharmaceutical uses from Gilman et al. (1985) or Thomson PDR<sup>®</sup> (2004).

<sup>5</sup>Only chemicals expected to be too volatile for the cytotoxicity assay system have "volatile" notations. BP=Boiling point. DEA (U.S. Drug Enforcement Agency) refers to Schedule II controlled substances. Chemicals with no "DEA" notation are expected to be under less strict control.

<sup>a</sup>RTECS<sup>®</sup> (MDL Information Systems 2002).

<sup>b</sup>Mouse