

Evaluation of the Nephrotoxicity of Complex Mixtures Containing Organics and Metals: Advantages and Disadvantages of the Use of Real-world Complex Mixtures

Jane Ellen Simmons,¹ Raymond S. H. Yang,² and Ezra Berman¹

¹Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina; ²Department of Environmental Health, Colorado State University, Fort Collins, Colorado

As part of a multidisciplinary health effects study, the nephrotoxicity of complex industrial waste mixtures was assessed. Adult, male Fischer 344 rats were gavaged with samples of complex industrial waste and nephrotoxicity evaluated 24 hr later. Of the 10 tested samples, 4 produced increased absolute or relative kidney weight, or both, coupled with a statistically significant alteration in at least one of the measured serum parameters (urea nitrogen (BUN), creatinine (CREAT), and BUN/CREAT ratio). Although the waste samples had been analyzed for a number of organic chemicals and 7 of the 10 samples were analyzed also for 12 elemental metals and metalloids, their nephrotoxicity was not readily predicted from the partial chemical characterization data. Because the chemical form or speciation of the metals was unknown, it was not possible to estimate their contribution to the observed biological response. Various experimental approaches, including use of real-world complex mixtures, chemically defined synthetic mixtures, and simple mixtures, will be necessary to adequately determine the potential human health risk from exposure to complex chemical mixtures. — *Environ Health Perspect* 103(Suppl 1):67–71 (1995)

Key words: metals, nephrotoxicity, complex mixtures, chemical interactions

Introduction

Humans are exposed either concurrently or sequentially to multiple chemicals by a variety of exposure routes. Given that most toxicologic evaluations involve assessment of single chemicals and that human exposure is typically to multiple chemicals, it is important to determine the circumstances under which the toxicity of chemical mixtures can be predicted based on knowledge of the toxicity of the individual components. Past and current studies on the non-cancer health effects of chemical mixtures or multiple chemical exposures have focused predominantly on simple mixtures

of two or three chemicals, describing the interaction and probing the underlying interactive mechanism. This mechanistic or bottom-up approach (1) has been dictated by the practical reality that it is impossible to assess the toxicity of all mixtures and multiple chemical exposures. To illustrate, evaluation of the interaction(s) of three chemicals at five different dose levels, including a zero and four nonzero dose levels for each chemical, in a full-factorial experiment (all possible dosing combinations of one, two, and three chemicals) would require 125 different treatment groups and assuming 6 or 10 animals per treatment group, 750 or 1250 animals, respectively. Ultimately, development of a mechanistic understanding of the behavior of simple mixtures will increase our ability to extrapolate to other dose levels, other exposure scenarios, other mixtures, and other species, including humans, leading to improvement(s) in the risk assessment of chemical mixtures.

The alternative to the bottom-up approach is the top-down approach in which research initiates with defining the toxicity of complex mixtures and is followed by determination of similarity to other mixtures of interest and identification of the toxic constituents (1). In comparison to simple mixtures, much less research on the noncancer health effects of complex

mixtures has been conducted and reported in the peer-reviewed literature. In this study, evaluation of the nephrotoxicity of complex industrial waste mixtures containing both organics and metals will be used to illustrate the advantages and disadvantages of the use of real-world complex mixtures in toxicologic evaluation of chemical interactions.

Ten samples of complex industrial waste were assessed in a multidisciplinary health effects study. The lethality, hepatotoxicity, and genotoxicity of these wastes have been reported previously (2–7). These studies were designed to aid in identification of toxic waste mixtures; evaluation and estimation of potential health risks; examination of biologic end points potentially suitable to screen complex waste mixtures of unknown toxicity; and assessment of the ability to predict biologic effects from partial chemical characterization data (8). A limited evaluation of the acute nephrotoxicity of these 10 waste samples was conducted in the same rats used for assessment of lethality and hepatotoxicity (4–6) but has not been reported to date.

Materials and Methods

The 10 waste samples were from the input stream of 6 hazardous waste incinerators (Table 1). The waste samples had been analyzed for a number of organic chemicals (Table 2) (9). This partial chemical

This paper was presented at the Joint United States–Mexico Conference on Fate, Transport, and Interactions of Metals held 14–16 April 1993 in Tucson, Arizona.

The authors gratefully acknowledge Dr. D. DeMarini and A. McDonald and thank Drs. L.S. Birnbaum, J. McKinney, D. DeMarini, and D. Thomas for thoughtful manuscript review.

The research in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Address correspondence to Dr. Jane Ellen Simmons, MD-74, HURL/U.S. EPA, Research Triangle Park NC 27711. Telephone (919) 541-7829. Fax (919) 541-5394.

Table 1. Physical description of complex waste mixtures.

Waste sample ^a	Physical state	Description
A	Liquid	Black, very thin oil
B	Liquid	Black, oily liquid
E	Liquid	Composite of organic wastes; thin, dark liquid
G ^{b,c}	Semi-liquid	Organic waste; biphasic, thick gray sludge with reddish-brown liquid
H ^c	Suspension	Aqueous waste; thin, gray slurry
J ^d	Liquid	Composite of organic wastes; thick, gray, liquid with suspended solids
K ^d	Liquid	Similar to J, but lighter in color and thinner
L and M ^e	Tar	Composite of organic wastes; black, thin, pourable tar
O ^e	Liquid	Composite of aqueous wastes; clear, watery liquid

^aTable from Simmons et al. (5). ^bSample G consisted primarily of a thick sludge that could not be dispersed by mixing; thus, only the liquid portion of G was tested. ^{c,d,e}Waste samples with the same superscript were from the same incinerator. The samples were from the input stream of six hazardous waste incinerators; two received waste from a single company (A,B); three accepted waste from a variety of industrial sources (E,J,K,L,M,O); and the waste source of one incinerator was not specified (G, H) (9).

Table 2. Concentrations (mg/g) of organic chemicals in complex waste mixtures.^a

Chemical	Waste sample ID									
	A	B	E	G	H	J	K	L	M	O
Aniline		14								
Benzene			0.3					46	58	<0.1
Benzylchloride	3.0									
Bis-(2-ethylhexyl)-phthalate		0.5	3.8					0.2	0.2	
Butylbenzylphthalate			0.3	0.5	<0.1	0.2	0.1			
Carbon tetrachloride ^b	68	44	6	4.4	<0.1	5.9	5.7	9.1	11.3	<0.1
Chlordane								<0.1	<0.1	
Chlorobenzene		4.1						0.4	0.5	<0.1
Chloroform	2.9			0.3	<0.1			0.1	<0.1	<0.1
Chloromethane		1.2								
Chlorophenylisocyanate		21								
Cresols						2	2.5			
<i>m</i> -Dichlorobenzene		2.3								
<i>o</i> -Dichlorobenzene		46								
<i>p</i> -Dichlorobenzene		59								
<i>cis</i> -1,4-Dichloro-2-butene	18									
<i>trans</i> -1,4-Dichloro-2-butene	59									
Diethylphthalate				1.3	0.2					
2,4-Dimethylphenol						0.5	2			
Hexachlorobutadiene								<0.1	<0.1	
Hexachlorocyclopentadiene								<0.1	<0.1	
Hexachloroethane	0.6									
Isophorone			0.1							
Methylene bromide								<0.1	<0.1	4.7
Methylene chloride	21							0.1	0.3	<0.1
Methyl ethyl ketone			9.7			18	38			
Naphthalene	<0.1		0.4	0.5	<0.1	0.4	0.5	<0.1	<0.1	
Phenol			1.0	1.7	1.8					
Phenylisocyanate		160								
Tetrachloroethylene	11		<0.1	9.8	1.6	7.9	8.1	<0.1	0.2	<0.1
Toluene	240		2.4	45	2.9	56	48	160	390	<0.1
1,2,4-Trichlorobenzene		0.3								
1,1,1-Trichloroethane	<0.1					24	16	0.3	0.2	<0.1
Trichloroethylene ^b	4	40	5.5	4.4	<0.1	8.1	7.8	8.3	10.3	<0.1
Water, %	1.8	0	3.2	48	95	21	23	4.7	5.3	96
Characterized mass, %	45	39	6	54	96	33	36	27	52	96

^aThese data were abstracted from the U.S. EPA (9). This table contains minor corrections of previously published versions. ^bFor the purposes of a study on the performance of incinerators (9), carbon tetrachloride and trichloroethylene were used as internal standards in samples B, E, G, J, K, L, and M.

characterization should not be viewed as indicative of the overall chemical composition of the samples as characterized mass ranged from a low of 6% for sample E to a

high of 96% for sample O. Additionally, 7 of the 10 waste samples had been analyzed for 12 elemental metals and metalloids (Table 3) (9). The details of the chemical

analyses have been described previously (9).

Adult, male F344 rats were exposed by gavage to 1 of the 10 waste samples, as described previously in greater detail (5). Rats were dosed in three sequential blocks, with concurrent controls in each block. Dosages, based on lethality at 5 ml/kg and on available sample volume, ranged from 0.5 to 5.0 ml/kg. Approximately 24 hr after dosing, rats were anesthetized, weighed, and bled from the abdominal aorta. The kidneys were excised and weighed. Serum was collected and frozen at -40°C until analyzed. Serum chemistry profiles were obtained commercially (Vet-Path, Teterboro, NJ) and included determination of the concentrations of urea nitrogen (BUN) and creatinine (CREAT). Feed (Purina Rodent Lab Chow No 5001, Ralston Purina, Checkerboard Square, MO) and tap water were available *ad libitum* except for the 16 to 18 hr prior to termination when feed but not water was withheld.

The data were subjected to Bartlett's test for homogeneity of variances (10). The criterion of significance for the homogeneity tests was $p \leq 0.001$ (11). The data from each block were analyzed by analysis of variance (12). When the results of this analysis indicated statistically significant differences between groups ($p \leq 0.05$), least-squares means were used to determine those treatment groups that varied significantly from the concurrent control ($p \leq 0.01$).

Results

Administration of 7 of the 10 tested samples resulted in mortality within 24 hr of dosing. The lethal potency of the samples varied greatly (Table 4) and they could be separated into three groups of descending toxicity (A,B,J,K) > (L,G,M) > (E,H,O). The deaths resulting from exposure to the waste samples and the limited sample volume available greatly limited our ability to do a full dose-response assessment of the target-organ toxicity of these mixtures. Four samples (A,E,G,M) increased absolute kidney weight, while 5 samples (A,E,G,L,M) increased relative kidney weight (Table 5). The increases in kidney weight ranged from approximately 8.5 to 10% at 5 ml/kg of samples E, G, and M to 25% at 1 ml/kg of sample A. The increases in relative kidney weight ranged from 7.8% at 2.5 ml/kg of sample L to 22% at 1 ml/kg of sample A. The BUN, CREAT, and BUN/CREAT data are summarized in Table 5. Exposure to only one of the 10 samples, A, resulted in an increase in serum BUN. Administration

Table 3. Concentrations (µg/g) of metals and metalloids in complex waste mixtures.^a

Chemical	Waste sample ID						
	A	G	H	J	K	L	M
Antimony	<12	61	<10	437	373	<24	<24
Arsenic	<24	<20	<20	<14	<14	<23	<23
Barium	<7	140	6	1160	1150	990	1100
Beryllium	<2	<1	<1	<1	<1	<1	<1
Cadmium	<5	6	<1	153	15	49	55
Chromium	<5	57	3	431	425	250	290
Lead	<19	150	<10	1830	1800	1200	1300
Mercury	<22	<10	<10	<4	<4	<50	<50
Nickel	68	7	2	26	27	<4	<4
Selenium	<470	<100	<100	<21	<21	<160	<160
Silver	<3	<1	<1	<1	<1	<1	<1
Thallium	<23	<20	<20	<9	<9	<22	<22

^aThese data were abstracted from U.S. EPA (9) by DeMarini et al. (3).

of 2 waste samples, E and G, resulted in increased serum CREAT, with both dose levels of sample E resulting in elevated CREAT. In contrast, 6 of the 10 waste samples resulted in significantly altered BUN/CREAT ratios relative to the respective block controls. BUN/CREAT was increased for sample A, due to increased BUN without a corresponding increase in CREAT. Decreased BUN/CREAT ratios were noted for samples E and G due to increased CREAT, while decreased BUN/CREAT for samples J, L, and O appear to reflect subtle, nonsignificant alterations in BUN and CREAT levels.

Discussion

It would be difficult to determine definitively the nephrotoxic potential of these waste samples because dose-response data are not

available due to limited sample availability and high mortality, histopathologic evaluation of the kidneys is not available, and serum BUN and CREAT are not notably sensitive to low levels of renal damage (13). Nonetheless, several interesting observations may be made. Absolute kidney weight has been observed to be a relatively sensitive indicator of nephrotoxicity for known nephrotoxicants (14). With nephrotoxicity defined as increased kidney weight (either absolute or relative) coupled with a significant alteration in at least one serum parameter, samples A, E, G, and L were nephrotoxic.

To avoid possible bias in the biological assessment of these waste samples, they were tested without prior regard for their chemical composition. Following toxicologic evaluation, the resulting toxicity was

Table 4. Lethality in rats administered complex waste mixtures.^a

Waste sample	Block	Dosage, ml/kg	No. dead / N	Mortality, %
Controls	1	0	0/8	0
	2	0	0/8	0
	3	0	0/8	0
A	3	1.0	1/6	17
	1	5.0	6/6	100
B	3	1.0	0/6	0
	1	5.0	6/6	100
E	2	2.5	0/4	0
	2	5.0	0/2	0
G	1	5.0	1/6	17
	3	5.0	0/6	0
J	3	0.5	0/6	0
	2	2.5	4/4	100
	2	5.0	2/2	100
K	3	0.5	0/6	0
	2	2.5	4/4	100
	2	5.0	2/2	100
L	2	2.5	0/4	0
	2	5.0	1/2	50
M	1	5.0	1/6	17
	0	2	5.0	0/6

^aTable from Simmons et al. (5).

compared to that which might have been expected based on knowledge of chemical composition. Comparisons of observed toxicity to predictions based on chemical composition are important, as an approach used to identify wastes as hazardous is based on partial chemical characterization (15). The organic and metal chemical profiles of the nephrotoxic waste samples were not readily distinguishable from those of the other waste samples. This can be illustrated by examination of the metal

Table 5. Renal effects in rats administered complex waste mixtures.^a

Waste sample	Block	Dosage, ml/kg	Body weight, g	Kidneys weight, g	Relative weight of kidneys, %	BUN, mg/dl	CREAT, mg/dl	BUN/CREAT
Controls	1	0	220.0 ± 5.8	1.67 ± 0.03	0.76 ± 0.02	21.1 ± 2.1	0.58 ± 0.05	36.9 ± 3.8
	2	0	201.5 ± 3.8	1.55 ± 0.06	0.77 ± 0.03	21.0 ± 2.6	0.60 ± 0.08	35.1 ± 2.4
	3	0	210.6 ± 5.1	1.60 ± 0.04	0.76 ± 0.01	22.6 ± 2.4	0.40 ± 0.08	57.6 ± 7.9
A	3	1.0	214.6 ± 7.9	2.00* ± 0.22	0.93* ± 0.07	79.5* ± 38 ^b	0.68 ± 0.19 ^b	110.6* ± 34.5 ^b
	1	5.0	—	—	—	—	—	—
B	3	1.0	209.0 ± 6.7	1.62 ± 0.07	0.78 ± 0.02	19.5 ± 1.9	0.58 ± 0.19	36.8 ± 13.8
	1	5.0	—	—	—	—	—	—
E	2	2.5	199.8 ± 3.2	1.60 ± 0.06	0.80 ± 0.03	22.0 ± 3.9	0.85* ± 0.24	26.4* ± 2.9
	2	5.0	200.2 ± 5.2	1.68* ± 0.003	0.84* ± 0.02	24.5 ± 3.5	0.85* ± 0.07	28.8* ± 1.8
G	1	5.0	216.0 ± 4.4	1.82* ± 0.08	0.84* ± 0.03	19.6 ± 2.8	0.78* ± 0.08	25.1* ± 2.6
H	3	5.0	211.5 ± 6.4	1.66 ± 0.11	0.78 ± 0.04	22.8 ± 2.8	0.47 ± 0.08	50.2 ± 11.1
J	3	0.5	207.6 ± 3.2	1.64 ± 0.07	0.79 ± 0.03	21.5 ± 3.6	0.65 ± 0.28	35.9* ± 9.0
	2	2.5	—	—	—	—	—	—
	2	5.0	—	—	—	—	—	—
K	3	0.5	211.9 ± 4.7	1.65 ± 0.06	0.78 ± 0.02	24.3 ± 2.9	0.67 ± 0.23	38.8 ± 8.5
	2	2.5	—	—	—	—	—	—
	2	5.0	—	—	—	—	—	—
L	2	2.5	193.5* ± 2.4	1.61 ± 0.06	0.83* ± 0.04	17.0 ± 2.0	0.68 ± 0.05	25.1* ± 1.2
	2	5.0	—	—	—	—	—	—
M	1	5.0	211.1 ± 9.1	1.83* ± 0.10	0.87* ± 0.06	24.5 ± 1.7 ^b	0.68 ± 0.10 ^b	36.7 ± 5.0 ^b
	0	2	202.9 ± 3.3	1.58 ± 0.05	0.78 ± 0.02	20.2 ± 3.3	0.65 ± 0.05	30.9* ± 2.7

^aData are reported as mean ± SD. ^bN shown in Table 4. *N = 4. *p ≤ 0.01.

profiles of several of the waste samples. Although J and K were among the most hepatotoxic of the waste samples (5), they produced little or no evidence of nephrotoxicity in this study. There is little difference in the elemental metal composition of these two apparently nonnephrotoxic samples and sample L, which produced evidence of nephrotoxicity.

It is important to note that only 7 of the 10 samples, including 3 of the 4 nephrotoxic samples, were analyzed for metals and that this analysis was for elemental metals. Because the chemical form of these metals is unknown, it is not possible to estimate the contribution of these metals to the observed biological response. Chemical form or speciation of a metal is an important determinant of absorption and distribution as well as of toxicity (16). For example, speciation influences either the pharmacokinetics, toxicity, or both of arsenic, chromium, lead, mercury, nickel, selenium, antimony, and barium (16,17). With mercury as an example, the gastrointestinal uptake of methylmercury compounds approaches 100% but for inorganic mercury compounds is less than 10% (18). Although neurotoxicity is produced by both methylmercury compounds and by elemental mercury, the signs and symptoms differ (18).

Although lack of knowledge of speciation is limiting, several interesting points may be noted. The metal concentrations in these wastes were low relative to those expected to produce nephrotoxicity. For example, an acute NOAEL in rats for cadmium nephrotoxicity has been reported to be 150 mg/kg (19). In contrast, the highest concentration of cadmium in the waste samples was 153 µg/g found in sample J. A 14-day study with mercuric chloride in F344 rats resulted in a NOAEL for renal effects of 0.93 mg/kg/day (20). It is also interesting to note that with the exception of selenium, the concentrations of metals in the nonnephrotoxic waste samples equaled or exceeded the concentrations in the nephrotoxic samples. Finally, nonadditive interactions, both antagonistic and synergistic, have been reported between various metals in the waste samples [for a review, see Krishnan and Brodeur (21)].

Although there were known nephrotoxic chlorinated organic chemicals in the waste mixtures, e.g., chloroform (CHCl₃) and hexachlorobutadiene (HCBD), they were not present at concentrations expected to produce toxicity. Sample A produced marked elevations in kidney weight and BUN and had the highest con-

centration of CHCl₃, 2.9 mg/g. This concentration of CHCl₃ is well below that producing overt nephrotoxicity in rodents following acute or subacute oral administration (22). Serum BUN levels in male Sprague-Dawley rats were not elevated 24 hr after oral gavage with 75 mg CHCl₃/kg (23). HCBD was present only in samples L and M and at very low concentrations, less than 0.1 mg/g. Male F344 rats exhibited increased serum BUN and kidney weight 24 hr after ip administration of 50 mg HCBD/kg, with substantially less toxicity seen in 63-day-old rats (the age closer to the rats used in the present study) than in 22-day-old rats (24). The strain dependence of HCBD nephrotoxicity in rodents is less prominent than age and sex differences; based on serum BUN 24 hr after administration of 100 mg HCBD/kg, male F344 rats are more susceptible than male Long Evans rats and at least as, if not more susceptible than male Sprague-Dawley and Alderly Park rats (25).

Although the level of chemical information available was greater than might be expected for most complex wastes, the characterization was far from complete. Less than 50% of the total mass was characterized for 6 of the 10 samples, with less than 60% of mass known for 8 of the 10 samples. In only two cases (samples H and O) was characterization greater than 90% complete and this was due to the fact that the water content of these samples was greater than 90%. Thus, the toxicity associated with these mixtures may be due to the presence of toxic components in the unidentified fraction. The presence of unidentified but highly toxic chemicals is of particular concern when trying to estimate or predict the toxicity of a mixture based on knowledge of some but not all of its component chemicals. Examples of toxicity being associated with a highly toxic contaminant that accounts for a minority of the total mass include 2,3,7,8-tetrachlorodibenzo-*p*-dioxin contamination of 2,4,5-trichlorophenol, hexachlorophene, chlorinated benzenes, and Agent Orange (26), and the significant contribution of nitroarenes to the mutagenicity of diesel emissions and carbon blacks (27).

While partial characterization of complex mixtures appears to be the rule rather than the exception (8), limitations in estimation of biologic activity from chemical composition information would exist even with complete knowledge of the chemical identity of a mixture. Toxicity data are lacking for many chemicals. Rat oral LD₅₀ data may be among the most commonly

available toxicity information; however, Simmons and Berman (6) found that 15% of the chemicals identified in these waste samples lacked even this basic information. Lack of toxicity data on component chemicals has been noted for mixtures as diverse as diesel exhaust (28) and those from Love Canal (29). In addition to sparse and/or insufficient toxicity information on many of the single chemicals present in mixtures, there are little data regarding potential interactions. Interactions among the many chemicals in these mixtures may include synergy, potentiation, antagonism, and additivity. Relative to the examination of nonadditive interactions in the liver, there has been little investigation of nephrotoxicity resulting from exposure to multiple chemicals.

Although the focus here is nephrotoxicity, it is important to note briefly that these samples have been evaluated also for lethality (as noted above), hepatotoxicity, mutagenicity in *Salmonella*, and genotoxicity in the prophage-induction assay. Qualitative responses are summarized in Table 6. As is readily discerned, the waste samples produced a different pattern of toxicity depending on the biologic end point. A limitation of biologic evaluation of hazardous waste samples is that toxicity or lack of toxicity in one assay may not be predictive of the effect in another assay system. For example, sample K was neither mutagenic, genotoxic, nor nephrotoxic but produced lethality and hepatotoxicity. Conversely, sample O appeared nontoxic in the *in vivo* mammalian assays but was mutagenic in *Salmonella*.

Work with real-world mixtures such as these 10 samples of complex industrial waste has the advantage of direct environmental relevance for that particular mixture. Disadvantages associated with toxicologic evaluation of real-world wastes include the difficulties in obtaining a homogeneous sample representative of a disposal site, leachate, or industrial waste stream due to changes in chemical composition between different areas of a waste disposal site as well as changes in chemical composition over time in the same area of a site and in the leachate from the site due to differences in the mobility and solubility of chemicals contained in the site (8). Additionally, waste streams from similar processes may vary in composition (30). An additional disadvantage is that chemical characterization of environmental samples is typically incomplete, greatly increasing the difficulty of extrapolation to other mixtures, even those whose known composition

Table 6. Summary of biological effects of complex waste mixtures.^a

Waste sample	Lethality	Hepatotoxicity ^b	Nephrotoxicity ^c	Salmonella mutagenicity ^d	Prophage induction ^e
A	+	+	+	+	+
B	+	+	○	○	+
E	○	+	+	○	NT
G	+	+	+	+	+
H	○	○	○	+	+
J	+	+	○	+	+
K	+	+	○	○	○
L	+	+	+	+	+
M	+	+	○	+	+
O	○	○	○	+	○

NT = not tested. ^aTaken, with exception of nephrotoxicity summary, from Simmons (8). ^bHistopathology was used as the criterion of hepatotoxicity. ^cIf a positive response was obtained for either absolute or relative kidney weight and at least one serum parameter (BUN, CREAT, BUN/CREAT), the summary response was considered positive. ^dIf a positive response was obtained for either the crude waste or its dichloromethane extract in either strain TA98 or strain TA100 with or without metabolic activation, the summary response was considered positive. ^eIf a significant response was obtained for the crude waste with or without metabolic activation, the summary response was considered positive.

REFERENCES

- U.S. EPA. Strategy for health effects research on chemical mixtures. ORD/HERL, 1992.
- DeMarini DM, Inmon JP, Simmons JE, Berman E, Pasley TC, Warren SH, Williams RW. Mutagenicity in *Salmonella* of hazardous wastes and urine from rats fed these wastes. *Mutat Res* 189: 205–216 (1987).
- DeMarini DM, Gallagher JE, Houk VS, Simmons JE. Toxicological evaluation of complex industrial wastes: implications for exposure assessment. *Toxicol Letters* 49:199–214 (1989).
- Simmons JE, DeMarini DM, Berman E. *In vivo* assessment of complex wastes: lethality, hepatotoxicity, and mutagenicity. In: Hazardous and Industrial Waste, Proceedings of the Twentieth Mid-Atlantic Industrial Wastewater and Hazardous Material Conference, 19–21 June 1988, Howard University, Washington (Varma MM, Johnson JH, eds). 1988;444–464.
- Simmons JE, DeMarini DM, Berman E. Lethality and hepatotoxicity of complex waste mixtures. *Environ Res* 46:74–85 (1988).
- Simmons JE, Berman E. Toxicity of complex waste mixtures: a comparison of observed and predicted lethality. *J Toxicol Environ Health* 27:275–286 (1989).
- Houk VS, DeMarini DM. Use of the microscreen phage-induction assay to assess the genotoxicity of 14 hazardous industrial wastes. *Environ Mol Mutagen* 11:13–29 (1988).
- Simmons JE. Toxicological evaluation of noncancer health effects of hazardous wastes. *Comments Toxicol* 3:469–491 (1990).
- U.S. EPA. Performance evaluation of full-scale hazardous waste incinerators. National Technical Information Service Publ no PB85–129500, 1984.
- Sokal RR, Rohlf FJ. *Biometry. The Principles and Practice of Statistics in Biological Research*, 2nd ed. New York:WH Freeman and Co., 1981.
- Anderson VL, McLean RA. *Design of Experiments: A Realistic Approach*. New York:Marcel Dekker, 1974.
- SAS Institute, Inc. *SAS/STAT User's Guide*. Version 6, 4th ed. Cary, NC:SAS Institute, 1989.
- Berndt WO, Davis ME. Renal methods for toxicology. In: *Principles and Methods of Toxicology*, 2nd ed. (Hayes AW, ed). New York:Raven Press, 1989;629–648.
- Kluwe WM. Renal function tests as indicators of kidney injury in subacute toxicity studies. *Toxicol Appl Pharmacol* 57:414–424 (1981).
- Greer LE. Definition of hazardous waste. *Hazard Waste* 1:309–322 (1984).
- Goyer RA. Toxic effects of metals. In: Casarett and Doull's *Toxicology: The Basic Science of Poisons*, 4th ed. (Amdur MO, Doull J, Klaassen CD, eds). New York:Pergamon Press, 1991;623–680.
- Gossel TA, Bricker JD. Metals. In: *Principles of Clinical Toxicology*, 2nd ed. New York:Raven Press, 1990;162–192.
- Manahan SE. *Toxicological Chemistry. A Guide to Toxic Substances in Chemistry*. Chelsea, MI:Lewis Publishers, 1989.
- ATSDR. Toxicological Profile for Cadmium. Atlanta:Agency for Toxic Substances and Disease Registry, April 1993.
- ATSDR. Toxicological Profile for Mercury. Atlanta:Agency for Toxic Substances and Disease Registry, May 1994.
- Krishnan K, Brodeur J. Toxicological consequences of combined exposure to environmental pollutants. *Arch Complex Environ Studies* 3:1–104 (1992).
- ATSDR. Toxicological Profile for Chloroform. Atlanta:Agency for Toxic Substances and Disease Registry, April 1993.
- Davis ME. Dichloroacetic acid and trichloroacetic acid increase chloroform toxicity. *J Toxicol Environ Health* 37:139–148 (1992).
- Kuo C-H, Hook JB. Effects of age and sex on hexachloro-1,3-butadiene toxicity in the Fischer 344 rat. *Life Sci* 33:517–523 (1983).
- Hook JB, Ishmael J, Lock EA. Nephrotoxicity of hexachloro-1:3-butadiene in the rat: the effect of age, sex and strain. *Toxicol Appl Pharmacol* 67:122–131 (1983).
- Menzer RE. Water and soil pollutants. In: Casarett and Doull's *Toxicology: The Basic Science of Poisons*, 4th ed (Amdur, MO, Doull J, Klaassen CD, eds). New York:Pergamon Press, 1991;872–902.
- Rosenkranz HS, McCoy EC, Mermelstein R. Microbial assays in research and in the characterization of complex mixtures. In: *Short-term Bioassays in the Analysis of Complex Environmental Mixtures. III* (Waters MD, Sandhu SS, Lewtas J, Claxton L, Chernoff N, Nesnow S, eds). New York:Plenum Press, 1983;103–138.
- Claxton LD. The integration of bioassay and physiochemical information for complex mixtures. In: *Short-term Bioassays in the Analysis of Complex Environmental Mixtures. III* (Waters MD, Sandhu SS, Lewtas J, Claxton L, Chernoff N, Nesnow S, eds). New York:Plenum Press, 1983;153–162.
- Goyer RA. Introduction and overview. *Environ Health Perspect* 48:1–2 (1983).
- Krag BL. Hazardous wastes and their management. *Hazard Waste Hazard Materials* 2:251–308 (1985).

is similar. Work with simple mixtures has the advantage that they can be readily used to test mechanistic hypotheses; elucidation of the underlying mechanism(s) results in greater confidence in extrapolation(s) to other interactions operating by the same mechanism(s). At the same time, work with simple mixtures has the disadvantage that most human exposure is thought to be to complex mixtures. Thus, improvements in the risk assessment of chemical mixtures will require a combination of approaches involving toxicological evaluation of real-world complex mixtures, chemically defined synthetic mixtures, and simple mixtures.