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Introductory paper

Seeking solutions to chemical mixtures challenges in public health

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Abstract

The Agency for Toxic Substances and Disease Registry (ATSDR) identifies people near hazardous waste sites who are at potential health risk because of their exposure to environmental chemicals. Nearly, 2000 chemicals have been associated with such sites. Residents of U.S. communities are potentially exposed to hazardous substances through air, soil, drinking water, and food. The agency has determined that more than 73 million people live within a 4-mile radius of waste sites. More than 14 million Americans live within 1 mile of a National Priorities List site, of which 11% are 7 years of age or younger, 12% are 64 years of age or older, 24% are women of childbearing age, and 25% are minorities. The lack of adequate environmental sampling and information on human exposures often restricts ATSDR's evaluation and assessment activities. Assessing human exposure with its attendant health risks and outcomes is complex because many populations have a wide range of reported illnesses, and generally exposures are to mixtures of chemicals. This prompted ATSDR to consider mixtures issues more in depth and to establish a formal mixtures assessment and research program in 1994. In this paper, we present an overview of the agency activities, the genesis, legislative mandates, and pertinence of the mixtures program including applied research and the development of methods for evaluating the impact of multiple-chemical exposure. On the basis of 20-year experience of evaluating and researching environmental chemical mixtures at waste sites, ATSDR convened the International Conference on Chemical Mixtures (ICCM) in 2002. The conference was supported by several federal agencies and scientific organizations and attended by international and national experts. The conference addressed broad topics such as prevalence of exposures to chemical mixtures, importance of interactions at environmentally relevant levels, validity of assuming additivity (dose or response) as default for mixtures assessment, and promising avenues in the three broad areas, viz., research, assessment, and computational tools.
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Keywords: Chemical mixtures; Hazardous waste; Exposure; Multiple chemical exposure

1. Introduction

ATSDR organizes peer-review panel meetings of expert scientists to address and resolve major issues encountered by the agency as well as national and international conferences. The International Conference on Chemical Mixtures (ICCM) was convened in Atlanta from September 10 to 12, 2002. Funding was supported by national and international organizations, including the Society of Toxicology (SOT), the U.S. Environmental Protection Agency (EPA), National Institutes of Environmental Health Sciences (NIEHS), Food and Drug Administration (FDA), National Institute of Occupational Safety and Health (NIOSH), International Joint Commission, and the Health Council of The Netherlands. ICCM brought together renowned scientists who use innovative techniques in research, assessment, and computational methods (the most useful areas for risk assessment methods development). The conference was attended by 150 expert scientists from 10 different countries. The following topics were addressed at the conference:

Abbreviations: ATSDR, Agency for Toxic Substances and Disease Registry; BBDR, biology based dose–response modeling; CERCLA, Comprehensive Environmental Response, Compensation, and Liability Act; CEPs, completed exposure pathways; HazDat, Hazardous Substance Release/Health Effects Database; HWS, hazardous waste sites; MRL, minimal risk level; NPL, National Priorities List; PBPK, physiologically based pharmacokinetic modeling; SAR, structure–activity relationships; SARA, Superfund Amendments and Reauthorization Act; U.S. EPA, U.S. Environmental Protection Agency

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- Prevalence of exposures to chemical mixtures.
- Importance of interactions at environmentally relevant levels.
- Validity of assuming additivity (dose or response) as default for environmental mixtures assessment.

The survey response from 90% of the ICCM participants indicated they believed population exposure to chemical mixtures is common to very common. More than 50% said chemical interactions at environmentally relevant exposure levels *are* important, and an additional 33% believed such exposure *may be* important. Several suggestions were made for further testing to obtain pertinent data to resolve this issue. These suggestions ranged from continuous testing to chronic testing of low-level or occupational-level exposures. More than 50% of survey participants agreed that additivity is a valid default approach for environmental mixtures assessment. Promising risk assessment avenues included dose–response analysis, the concept of interaction threshold, and evaluation of real-world situations. Promising research methods included enhancing ATSDR's chemical-interaction database, mechanistic studies, and use of post-genomic technologies. Promising computational tools included biology based dose–response modeling to understand genetic level changes and biological-process modeling. The articles in this issue of *Environmental Toxicology and Pharmacology* will express the scholarly ideas and opinions of the conference organizers and attendees and will further explain the pertinent issues that were briefly answered through the ICCM survey. The risk-assessment process includes hazard identification, dose–response assessment, exposure assessment, and risk characterization. Exposure to chemical mixtures has an effect on each of these aspects of risk assessment, and such exposure needs to be understood well before meaningful environmental assessments can be made.

2. Legislative mandates

In 1980, Congress created the ATSDR to implement health-related sections of federal laws that protect the public from hazardous wastes and environmental spills of hazardous substances. The Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA), commonly known as Superfund, provided the Congressional mandate to remove or clean up toxic substances at abandoned and inactive hazardous waste sites and to provide federal assistance during toxic emergencies. As the lead agency within the U.S. Public Health Service for implementing the health-related provisions of CERCLA, ATSDR is required to assess the presence and nature of health hazards at specific Superfund sites, to help prevent or reduce further exposure and the illnesses that result from such exposures, and to expand the knowledge based on health effects from exposure to hazardous substances.

With the passage of the Superfund Amendments and Reauthorization Act of 1986, ATSDR received additional responsibilities in environmental public health. This act broadened ATSDR's responsibilities in the areas of public health assessments, establishment and maintenance of toxicologic databases, information dissemination, and medical education. This statute further directed ATSDR, where feasible, to develop methods to determine the health effects of chemical mixtures (substances combined with other substances) that are commonly found at hazardous waste sites. This prompted ATSDR to initiate, coordinate, and conduct chemical mixtures research that would advance existing methods for chemical mixtures health assessment (Au and Falk, 2002).

3. Hazardous waste sites and human health concerns

One of ATSDR's primary goals is to identify people who are at potential health risk because of their exposure to environmental chemicals at more than 1000 waste sites that the EPA has identified and placed on its National Priorities List (NPL) (ATSDR, 2001, 2003; Johnson and De Rosa, 1995). Another 40,000 uncontrolled waste sites pose a potential threat for human exposure to chemicals mixtures. Nearly, 2000 chemicals have been associated with such waste sites, and ATSDR reviews and evaluates the toxicity of these chemicals to determine the public health risk. The agency has determined that more than 73 million people live within a 4-mile radius of waste sites. Across the nation, more than 14 million people live within 1 mile of an NPL site, of which 11% are 7 years of age or younger, 12% are 64 years of age or older, 24% are women of childbearing age, and 25% are minorities. Of the waste sites ATSDR has evaluated, 37% had either completed or potentially completed exposure pathways (CEPs). Although numerous chemicals might occur in the environment and often be encountered at hazardous waste sites, not all chemicals are of actual public health concern. A method to identify chemical mixtures that are of actual public health concern has been linked to CEPs at various hazardous waste sites. A CEP evaluation identifies and characterizes the following five elements: source of contamination, environmental medium, point of exposure, route(s) of exposure, and a receptor population (ATSDR, 1992; Mumtaz et al., 1994a,b). A CEP occurs when all five of these elements are present *and* the contaminant source is linked to a receptor population. Should a CEP exist in the past, present, or future, the population is considered exposed. A potential exposure pathway exists when one or more of the five elements are missing or if modeling is performed to replace real sampling data (e.g., modeled groundwater data using soil or other groundwater data levels). An analysis using the agency's comprehensive, hazardous substance release/health effects database (HazDat) showed that approximately 37% of the NPL sites ATSDR has evaluated to date, had at least one chemical identified in a CEP. At these sites, exposures to these chemicals are as follows: 91% through groundwater, 46% through con-

taminated soil, and 14% through contaminated biota. The top five chemicals on ATSDR's CEP site count report were trichloroethylene (TCE), lead, tetrachloroethylene, arsenic, and benzene.

The total body burden of exogenous chemicals found in the human populations living in the vicinity of such sites is due partly, to potential complex chemical mixtures found in the environmental media (De Rosa et al., 1991). Exposure may also include environmental, occupational, and personal agents. Concurrent exposure to chemicals, such as welding fumes, indoor air pollutants, tobacco smoke, alcohol, prescription and nonprescription drugs, and cosmetics makes health assessment of exposure to waste-site chemicals a more complex task (OSHA, 1993). Voluntary exposures such as those found in occupational settings can involve exposures to relatively high chemical concentrations for a long duration and can be well-defined and quantified (NIOSH, 1996). Conversely, involuntary exposures from waste sites may be infrequent, of unknown duration, and at low concentrations making them difficult to characterize and quantify. ATSDR's findings have pointed to health threats faced by people living near hazardous waste sites who drink contaminated water, eat contaminated fish, breath toxic fumes, or are otherwise exposed to hazardous substances (ATSDR, 2003). ATSDR identifies the need for (1) health education in a community, (2) health studies to be conducted, or (3) issuance of a public health advisory to recommend immediate actions to reduce, prevent, and eliminate exposure.

In this 23rd anniversary of its creation by CERCLA, ATSDR pursues its mission, "... to serve the public by using the best science, taking responsive public health actions, and providing trusted health information to prevent harmful exposures and disease related to toxic substances." Based on the current science, this mission consists of community and site activities that prevent or reduce exposure and adverse human health effects and diminished quality of life associated with exposure to hazardous substances from waste sites, unplanned releases, and other sources of pollution present in the environment (ATSDR, 1993).

In the wake of the tragedy of September 11, 2001 ATSDR has new roles in terrorism preparedness and response activities regarding exposure to environmental chemicals. ATSDR collaborates with EPA, the National Response Team (with its 16-member agencies), Federal Bureau of Investigation, the Federal Emergency Management Agency/Department of Homeland Security, and the state health departments. Even though ATSDR's activities have been expanded from its initial mandates, the new activities still fit within the overall ATSDR mission to prevent or reduce the harmful effects of exposure to hazardous substances on human health and quality of life.

ATSDR's major goals are to: (1) prevent ongoing and future exposures and resultant health effects from hazardous waste sites and releases, (2) determine human health effects associated with exposures to Superfund-related priority hazardous substances, (3) mitigate the risks of human health effects at toxic waste sites with documented exposures, (4) build and enhance effective partnerships, and (5) promote effective and efficient agency management.

To achieve these goals, ATSDR has developed an applied research program. The agency seeks answers to important questions about exposure to hazardous substances and health outcomes. Through evaluation and interpretation of existing data on substances found at sites on the NPL, priority data needs for high-ranking substances are identified in ATSDR's toxicological profiles. In cooperation with the National Toxicology Program, ATSDR uses several mechanisms to fill priority data needs; these include (1) toxicologic testing of chemicals in collaboration with EPA (under the provisions of the Toxic Substances Control Act); (2) private-sector voluntarism; (3) a partnership with minority-health-professions schools; and (4) a chemical mixtures assessment and research program.

4. Chemical mixtures research

To meet the mandates of the agency, a strategic plan was developed for a chemical mixtures program in 1994. Before implementation, this proposed program underwent agency-wide review and was approved by the agency's senior managers and its Board of Scientific Counselors. Subsequently, the plan was presented to and endorsed by EPA and the NIEHS during discussions of the Tri-Agency Superfund Applied Research Committee.

The primary objectives of the mixtures program are to develop rules that can be generalized for the toxicity assessment of a variety of chemical mixtures to which humans are often exposed and to advance the methods for evaluating the potential for joint toxic action. The generation and analysis of relevant data from a broad range of sources are being used to achieve these objectives. The following three areas of research highlight this program: (1) identification and listing of priority chemical mixtures that will reflect the combinations of chemicals in the environment that may be of real-life concern, (2) data analysis and assessment of existing information so as to evaluate the toxicity of identified mixtures on public health, and (3) academically based laboratory research and in vivo and in vitro toxicological testing of mixtures of concern if data are not adequate. Joint action is estimated by using various methods including computational techniques, such as physiologically based pharmacokinetic (PBPK) modeling and structure–activity relationship techniques.

The first component of the mixtures program is identification and listing of priority chemical mixtures through a trend analysis of data in the HazDat (ATSDR HazDat, 2003). HazDat is a comprehensive, database that contains detailed environmental contamination data from specific waste sites for which ATSDR has conducted public health assessments, prepared health consultations, or provided responses to emergencies involving releases of toxic substances into commu-

nity environments. This database also contains information abstracted from ATSDR toxicological profiles on more than 200 substances frequently encountered at waste sites. Typically, HazDat is used to extract data on (1) the kinds of contaminants released from a specific waste site, (2) the environmental media that are most affected by such contaminants, and (3) the site-specific pathways by which people are exposed to such contaminants.

To date, trend analyses have been performed for frequently co-occurring chemicals in air, water, and soil that are collected at or near waste sites (Johnson and De Rosa, 1995; De Rosa et al., 1996; Fay and Mumtaz, 1996). An initial analysis (Fay and Mumtaz, 1996) of frequencies of common binary and ternary combinations of chemicals in water, soil, and air for all sites on the NPL revealed that some of the most common environmental contaminants are found in combinations (Table 1). Emphasis is now directed towards trend analysis and explicit identification of chemical mixtures in the CEPs. The analyses for binary chemicals found in CEPs reveal that the highest ranked chemical combinations remain somewhat the same even though their percentages might decrease (De Rosa et al., 2004). Thus, trichloroethylene and tetrachloroethylene remains the top binary combination in water. Through these types of trend analyses, various combinations of chemicals have been identified to which human populations can be potentially exposed.

The second component of the mixtures program is the assessment of the joint toxic action of chemicals. ATSDR develops a series of interaction profiles that are used to evaluate joint toxic action of the most commonly co-occurring contaminants identified through trend analysis (Table 2). These interaction profiles provide a systematic way to evaluate mixtures that are of special interest to public health by presenting data on the toxicity of individual chemicals and data on various combinations of the contaminants (ATSDR, 2002b–g). These mixture toxicity assessments are based on individual component toxicity and available interaction data, which distinguish these assessments from other toxicologic reviews of chemicals. Two interaction profiles have been developed to evaluate the potential toxicity of persistent contaminant-

Table 1
Single substance and combinations frequencies

Number	Sites (%)	Single substance	Sites (%)	Binary pairs		Sites (%)	Trinary (tertiary) combinations		
Water									
1	42.4	TCE	23.5	TCE	Perc	11.6	1,1,1-TCA	TCE	Perc
2	38.4	Lead	18.9	Lead	Chromium	10.6	Benzene	TCE	Perc
3	27.3	Perc	17.9	1,1,1-TCA	TCE	10.6	Lead	Cadmium	Chromium
4	25.8	Benzene	17.3	TCE	Lead	9.8	1,1,1-TCA	1,1-DCA	TCE
5	25.8	Chromium	17.2	Lead	Cadmium	9.7	Lead	Arsenic	Cadmium
6	23.9	Arsenic	17.0	Benzene	TCE	9.7	TCE	Perc	Lead
7	20.8	1,1,1-TCA	16.3	Lead	Arsenic	9.6	Lead	Arsenic	Chromium
8	20.3	Toluene	14.5	TCE	Trans-1,2-DCE	9.4	Benzene	TCE	Toluene
9	19.8	Cadmium	13.6	TCE	Toluene	9.3	TCE	Perc	Trans-1,2-DCE
10	17.7	MeCl*	13.5	Benzene	Lead	9.1	TCE	Lead	Chromium
Soil									
1	37.7	Lead	20.5	Lead	Chromium	12.0	Lead	Cadmium	Chromium
2	25.3	Chromium	17.8	Lead	Arsenic	11.6	Lead	Arsenic	Chromium
3	23.0	Arsenic	17.6	Lead	Cadmium	10.9	Lead	Arsenic	Cadmium
4	19.7	Cadmium	13.3	Arsenic	Chromium	8.4	Arsenic	Cadmium	Chromium
5	19.1	TCE	12.9	Cadmium	Chromium	8.1	Lead	Nickel	Chromium
6	16.0	Toluene	11.6	Arsenic	Cadmium	7.9	Lead	Chromium	Zinc
7	14.8	Perc	10.9	TCE	Perc	7.7	Lead	Copper	Zinc
8	13.6	PCBs	10.9	Lead	Zinc	7.6	Toluene	Lead	Chromium
9	13.0	Xylenes	10.4	Ethylbenzene	Toluene	7.5	Ethylbenzene	Toluene	Xylenes
10	12.8	Ethylbenzene	10.4	Lead	Nickel	7.5	Lead	Nickel	Cadmium
Air									
1	6.0	Benzene	3.5	Benzene	Toluene	2.2	Benzene	TCE	Perc
2	4.7	Toluene	2.7	Benzene	TCE	1.9	Benzene	Ethylbenzene	Toluene
3	3.8	TCE	2.6	Benzene	Perc	1.8	Benzene	Toluene	Perc
4	3.4	Perc	2.6	TCE	Perc	1.8	Benzene	TCE	Toluene
5	3.1	1,1,1-TCA	2.3	Toluene	Perc	1.8	TCE	Toluene	Perc
6	2.6	Lead	2.1	Ethylbenzene	Toluene	1.4	1,1,1-TCA	Toluene	Perc
7	2.5	Ethylbenzene	2.1	TCE	Toluene	1.4	1,1,1-TCA	TCE	Perc
8	2.4	MeCl*	1.9	1,1,1-TCA	TCE	1.3	Benzene	1,1,1-TCA	Perc
9	2.4	Xylenes	1.9	Toluene	Xylenes	1.3	Benzene	Toluene	Xylenes
10	1.8	Chloroform	1.9	1,1,1-TCA	Perc	1.3	1,1,1-TCA	TCE	Toluene

MeCl, methylene chloride; PCBs, polychlorinated biphenyls; Perc, perchloroethylene (tetrachloroethylene); 1,1,1-TCA, 1,1,1-trichloroethane; TCE, trichloroethylene; Trans-1,2-DCE, trans-1,2-dichloroethylene; 1,1-DCA= 1,1-dichloroethane. Frequencies of single substances and combinations as percent occurrence at the 1,188 sites surveyed. Note the predominance of inorganics in soil and volatile organics in air, with a mix of the two in water (Fay and Mumtaz, 1996).

Table 2
List of interaction profiles developed by ATSDR for specific environmental mixtures of public health concern

Arsenic, cadmium, chromium, and lead
Benzene, toluene, ethylbenzene, xylene (BTEX)
Lead, manganese, zinc, and copper
Persistent chemicals in breast milk (polychlorinated biphenyls (PCBs), chlorinated dibenzo-*p*-dioxins (CDDs), DDE, hexachlorobenzene, and methylmercury)
Persistent chemicals in fish (polychlorinated biphenyls (PCBs), chlorinated dibenzo-*p*-dioxins (CDDs), DDE, hexachlorobenzene, and methylmercury)
1,1,1,-Trichloroethane, 1,1,-dichloroethane, trichloroethylene (TCE), and tetrachloroethylene (PCE)
Cesium, cobalt, strontium, polychlorinated biphenyls (PCBs), trichloroethylene (TCE)
Arsenic, hydrazine, jet fuels, strontium, trichloroethylene (TCE)
Cyanide, fluoride, nitrate, and uranium

mixtures exposure through consumption of fish and human milk (ATSDR, 2002b,c). The featured chemicals, polychlorinated biphenyls (PCBs), chlorinated dibenzo-*p*-dioxins (CDDs), dichlorodiphenyl dichloroethane (*p*,*p*′-DDE), hexachlorobenzene, and methylmercury, frequently occur together in water, sediment, and fish from the North American Great Lakes and also occur in other dietary components, including fish from other parts of the world (e.g., the Baltic Sea), human milk, dairy products, and meat. The mixture assessment in these interaction profiles is based on the weight-of-evidence (WOE) methodology (Mumtaz and Durkin, 1992). This evaluation consists of several components, including toxicologic significance (target organ/dose data), and mechanistic understanding (toxicokinetic/toxicodynamic).

In summary, these interaction profiles indicate that only limited evidence is available to support the possible existence of greater-than-additive or less-than-additive joint actions for a few pairs of contaminants (Table 3) as follows: (1) hexachlorobenzene potentiation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) reduction of body and thymus weights (Li et al., 1989); (2) PCB antagonism of TCDD immunotoxicity and TCDD developmental toxicity (Bannister et al., 1987; Davis and Safe, 1989; Haake et al., 1987), and (3) synergism between PCBs and methylmercury in disrupting regulation of brain levels of dopamine that may influence neurological function and development (Bemis and Seegal, 1999). For other chemical pairs, additive joint action at shared targets of toxicity was either supported by data for a few pairs or recommended as a public-health-protective assumption because adequate data to assess joint toxic action were not available. In general, overlapping targets of toxicity for these five components provide strong support for the plausibility of joint toxic action, but a notable lack of studies exists to characterize the modes of joint toxic action. Because limited data are available from whole mixture studies to characterize health hazards of mixtures of CDDs, hexachlorobenzene, *p*,*p*′-DDE, methylmercury, and PCBs, additive joint toxic action based on the component-based approach is often employed. In the first step, a target-organ toxicity dose (TTD) modification of

Table 3
Selected examples of interaction data documented in ATSDR interaction profiles

Joint toxic action of lead and zinc:
Good mechanistic and fair toxicological significance data establish that the neurotoxic effects of lead will be decreased due to the presence of zinc. Also, good mechanistic as well as toxicological significance data establish that the hematologic effects of lead will also be decreased due to the presence of zinc (<IB neurologic effects, <IA hematological).
Joint toxic action of lead and copper:
Good mechanistic and weak toxicological significance data establish that the neurotoxic effects of lead will be decreased due to the presence of copper. But good mechanistic and fair toxicological significance data establish that the hematologic effects of lead will also be decreased due to the presence of copper (<IC neurologic effects, <IB hematological).
Joint toxic action of lead and cadmium:
Fair mechanistic and good toxicological significance data establish that the renal effects of lead will be decreased due to the presence of cadmium. But fair mechanistic and good toxicological significance data establish that the testicular effects of lead will be increased due to the presence of cadmium (<IIA renal, >IIA testicular).
Joint toxic action of TCDD and PCBs:
Weak mechanistic and fair toxicological significance data establish that the immune suppressive effects of TCDD decreased due to the presence of PCBs. Also, weak mechanistic as well as weak toxicological significance data establish that the developmental effects of TCDD will be decreased due to the presence of PCBs (<IIIB immune suppression, <IIIC developmental).
Joint toxic action of TCDD and hexachlorobenzene (HCB):
Weak mechanistic and good toxicological significance data establish that the increase in whole body and thymus weight due TCDD will increase due to the presence of HCB (>IIIA body and thymus weight).
Joint toxic action of TCDD and *p*,*p*′-DDE:
Weak mechanistic and weak toxicological significance data establish that the antiandrogenic effects of TCDD will not change due to the presence of *p*,*p*′-DDE. Also, weak mechanistic as well as weak toxicological significance data establish that the developmental effects of TCDD will be decreased due to the presence of PCBs (=IIIC antiandrogenic effects).
Joint Toxic action of TCDD and *p*,*p*′-DDE:
Weak mechanistic and weak toxicological significance data exists that establishes that the antiandrogenic effects of TCDD will not change due to the presence of *p*,*p*′-DDE (=IIIC antiandrogenic effects).

the hazard index (HI) approach is used to conduct a screening level analysis for exposure-based assessments of noncancer health hazards. Alternatively stated, HIs are computed on an organ-specific basis, assuming that target-organ toxicities are biologically independent. TTDs for several toxicity targets have been derived for each of the components, including TTDs for hepatic, endocrine, immunological, reproductive, developmental, and neurological effects. For assessment of cancer risks from joint toxic action of the mixture, a similar component-based approach was recommended consisting of multiplying intakes of the chemical components by EPA cancer slope factors and summing the resultant risk estimates.

Another interaction profile was developed for mixtures of benzene, toluene, ethylbenzene, and xylenes (BTEX) that

frequently occur together at hazardous waste sites (ATSDR, 2002e). Combinations of these chemicals are among the most frequently found binary mixtures in CEPs at hazardous waste sites. Environmental media contaminated with BTEX include air, water, and soil. When contaminated groundwater is used as household water, BTEX components can volatilize into indoor air. In addition, contamination of groundwater and subsurface soil can result in these chemicals migrating into basements as soil gas. Whole BTEX mixture toxicity data are lacking, and information pertaining to toxic interactions among the BTEX components is essentially limited to data on a few binary mixtures of the chemicals. However, predictions from PBPK modeling studies, when used in conjunction with mechanistic, interaction, and toxicity information on the components, provide a sufficient basis for assessing the joint toxic action of the whole mixture in humans. PBPK models have been developed for binary, ternary, and quaternary mixtures of BTEX components in humans as well as rats (Haddad et al., 1999, 2000; Purcell et al., 1990; Tardif et al., 1993a,b, 1995, 1997). Similarly, a PBPK model was developed that predicts blood levels of the BTEX chemicals in rats (Haddad et al., 1999).

The third component of the mixtures program is ATSDR-supported targeted research to fill certain data gaps needed for assessment. Experimental studies that consist of a range of toxicity testing and research efforts have been carried out in cooperation with the NIEHS, private-sector groups, and academic institutions. This collaborative effort helps support development of chemical-mixtures assessment methods. These activities utilize limited in vivo and in vitro methods and available assays to conduct toxicologic research without compromising the sensitivity or specificity. Recently developed innovative techniques are included. Advantage is taken of modeling methods, such as PBPK and biologically based dose–response modeling. All of these efforts interrelate and are important for mixtures assessments and good public health practice. The trend analyses, toxicological testing, and development of computational models are iterative directed research activities that can define, suggest, and help prioritize issues needing to be resolved. ATSDR's chemical mixtures program allows scientists to pursue various aspects of chemical mixtures research, including the following: identifying environmental chemical mixtures that affect public health; evaluating the potential for human exposure; identifying various endpoints that would be affected; evaluating target organs that would be affected; studying the mechanisms of action, progression, and repair; identifying biomarkers (specific and generic) that would allow the determination of the health of an organism; and developing qualitative and quantitative health assessment methods for assessing multiple health effects (see Table 4).

ATSDR has developed several guidance manuals in collaboration with a broad range of partners, including EPA, the Toxicology and Nutrition Office (TNO) (The Netherlands), NIEHS, and academia-based research, to accomplish the goal of evaluating exposures to environmental contaminants and resultant human health effects by using a consistent framework (ATSDR, 1992, 1995, 2002). These guidance manuals can be used by health assessors and other health professionals to assess the potential joint toxicity of environmental contaminants and to determine if further follow-up activities or studies may be warranted. Joint toxicity assessment is a formidable task because all the possible chemical mixtures to which human beings are potentially exposed cannot be experimentally tested. Also, the literature on human health effects from chemical mixtures is limited and often suffers from lack of good exposure assessments. This dilemma demands a critical analysis and synthesis of relevant data to identify rules that can be generalized for use in site-specific assessments.

Table 4
Experimental mixtures program for research and development of methods for the joint toxicity assessment of environmental mixtures

PI: Ray Yang, Ph.D., Colorado State University (CSU)
Project title: Application of toxicogenomics to hazard identification of chemical mixtures
CSU researchers are developing an efficient hazard identification approach for carcinogenic potential of chemical mixtures by utilizing toxicogenomics. CSU is using cDNA microarray "biosignatures" as a predictive analytical tool. Implementation of microarray screening of xenobiotics for carcinogenic activity should greatly facilitate cost-effective hazard identification, and potentially, allow more "high throughput" risk assessment.
PI: Subhash Basak, Ph.D., University of Minnesota (UM)
Project title: Predicting toxicity of chemical mixtures using structural genomics and proteomics
UM researchers are developing computational approaches for the prediction of mixture toxicity using a set of halocarbons as the first experimental set. Some of the halocarbons to be tested include: vinyl chloride, chloroform, trichloroethylene, tetrachloroethylene. Novel sets of biodescriptors for the quantification of proteomic maps are being developed. These biodescriptors will be used to help elucidate modes of actions of the toxicants.
PI: Harihara Mehendale, Ph.D., University of Louisiana at Monroe (ULM)
Project title: Development of Research Methods for the Joint Toxicity Assessment of Environmental Mixtures
ULM researchers are investigating the health effects of mixtures of the common environmental contaminants TCE, PERC, TCA, and CHCl3. The toxicokinetics, toxicodynamics, genomic and proteomic profiles will be studied following administration of these chemicals individually and as mixtures. PBPK models will be constructed using laboratory-generated data. The proposed study implements sensitive, state-of-the-art techniques to evaluate toxicity and adverse health outcomes in response to chemical mixture exposure.
PI: Jeff Fisher, Ph.D., University of Georgia (UGA)
Project title: Development of a biologically-based model for chemical mixture-induced perturbations of the pituitary/hypothalamus-thyroid axis
UGA researchers are developing and validating physiologically-based pharmacodynamic (PBPD) models of the pituitary/hypothalamus-thyroid (P/H-T) axis for rats and humans to reliably predict the impact of specific chemical mixtures on the thyroid. Models for perchlorate, hexachlorobenzene, and PCB 126 will be constructed and will be used to predict and evaluate the effects of these particular chemical mixtures on thyroid hormone levels in adult humans.

The draft *Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures* (ATSDR, 2002a) is a document that provides a summary of issues related to health and toxicity assessment of chemical mixtures. This draft manual includes a discussion of the latest tools and methods that are useful for assessing mixtures toxicity and conducting health assessments. Alternative approaches are provided for evaluating the joint toxicity of chemical mixtures encountered at hazardous waste sites. This guidance builds on accepted concepts of mixtures toxicity that have been supported by years of animal research (usually at high doses) but have been adopted by various federal agencies and offices, including EPA, NIOSH, and FDA (EPA, 1986, 1989; NIOSH, 1996; Yang, 1994). The draft guidance recommends the use of a WOE methodology for evaluation of chemical interactions. This methodology allows the identification of data gaps and suggests strategically targeted applied research needed to advance the methods development. Several tiered steps are included leading to the most plausible evaluation of possible health risks and using the most current risk assessment tools. For example, using toxicity and exposure data available on the specific mixture of concern is generally recommended. If data on the mixtures of concern are not available, then using data on similar or related mixtures is recommended. The third option offered is using data available on the components of the mixture. In the guidance, methods that use the component data to evaluate the whole mixture include the following: the HI approach, target-organ toxicity dose modification of the HI approach, WOE modification of the HI method, and toxicity equivalency and relative potency methods.

The HI approach is the most generally accepted method used to screen for the toxicity of chemical mixtures. This approach approximates the toxicity index that would have been calculated had the mixture itself been tested. However, because of resource limitations, most environmental mixtures have not been tested. When this approach is used, all plausible candidates for the critical effects of the mixture are usually considered by calculating, if possible, organ-specific hazard indices. Then the role is considered of potential interactions that could modify the expected outcome when chemicals occur together. Integration of the knowledge and insights gained about chemical interactions and the actions of the chemical components of a mixture becomes part of the WOE for interactions. Toxicological interactions among mixture components can either increase or decrease the apparent toxicity of a mixture. This step relies on scientific judgment, based on empirical observations and mechanistic considerations, that categorizes the most plausible nature of potential interactions for a given exposure scenario. Currently, an ATSDR workgroup has been charged to evaluate the utility of the HI approach in real field settings and assist in risk communication with communities.

In recent years, ATSDR has addressed public health issues involving hazardous waste sites by directly dispersing more than 50% ($37 million) of its total budget through contracts, grants, cooperative agreements, and interagency agreements to support environmental health and research programs in state and local health departments, educational institutions, and other organizations serving public health. Because of ATSDR's 20-year experience in public health evaluation, the agency is in a strong position to engage in a long-term research program. The agency plays a unique leadership role in identifying and compiling new knowledge base that is applicable to public health. ATSDR's focus and strengths reside in community public health practice and support services. The agency serves as the lead federal agency for Superfund public health activities; environmental public health practice; support for states and tribes in various activities; and identifying, evaluating, and intervening to reduce exposures and adverse human health outcomes.

5. Agenda for public health and environmental research (APHER)

With the involvement of many organizational partners and constituents, ATSDR has developed the agenda for public health and environmental research (APHER), a 10-year research program (Spengler and Falk, 2002). APHER was developed after consideration of various issues and discussions with partners and collaborators to address and improve the agency's effectiveness in meeting its mission and goals. The activities defined in APHER fit within the agency's critical mission to prevent or reduce the harmful effects of exposure to hazardous substances on human health and quality of life. ATSDR's major goals are to (1) prevent ongoing and future exposures and resultant health effects from hazardous waste sites and releases, (2) determine human health effects associated with exposures to Superfund-related priority hazardous substances, (3) mitigate the risks of human health effects at toxic waste sites with documented exposures, (4) build and enhance effective partnerships, and (5) promote effective and efficient agency management. The agency supports both intramural and extramural research using various funding mechanisms, including contracts, cooperative agreements, and grants. APHER will highlight the following six focus areas during 2002–2010: exposure assessment, chemical mixtures, susceptible populations, community and tribal involvement, evaluation and surveillance of health effects, and health promotion and intervention. ATSDR's Superfund-mandated research and public health activities will be enhanced through these six focus areas and will help guide research to fill critical data and information gaps (Mumtaz et al., 1991)

Twenty-six initial research projects within the six focus areas were proposed to address several important and recurring themes that include partnering, community involvement, and consideration of social, cultural, and biological issues. Numerous opportunities exist for working collaboratively in research with various governmental partners, professional associations, universities, nongovernmental organizations, affected citizens, community groups, and Native

American tribes. This agenda will be used to guide, coordinate, and monitor the development and implementation of applied-research activities supported either by ATSDR or through extra funding in collaboration with other partners. The agenda will be a useful planning and communications tool, will foster collaboration on cross-cutting areas of research within and outside the agency, and will provide a renewed focus on using research to improve ATSDR's public health services to communities and tribes.

In developing the agenda, ATSDR determined the priority of ongoing and new research projects according to criteria that address a problem of public health or scientific importance. Many of the following factors were used to determine the importance: (1) the toxicity of contaminant(s), (2) frequency of contaminant or mixture found at sites, (3) number and size of exposed populations, (4) priority data needs identified in an ATSDR toxicological profile, (5) biological and toxicological prediction of specific health outcomes, (6) severity of outcomes in terms of mortality or disability, or estimated number of persons expected to develop an adverse outcome.

In finalizing the agenda, emphasis was placed on the need to involve communities that have documented environmental hazards and exposures; using feasible and appropriate methods that reflect the best scientific practice; addressing and improving agency effectiveness in meeting its mission and goals; focusing on children, minorities, and other susceptible populations whenever possible; collaborating with communities and Native American tribes to actively involve them in the research; conducting research that is not redundant or does not duplicate work already completed or in progress.

Under APHER, the goal of the chemical mixtures focus areas will continue to improve the understanding of the toxicity of common mixtures of hazardous substances found in contaminated environmental media at waste sites and to develop methods and approaches for assessing their joint toxicity. Research projects that will be supported through this focus area will include the following:

- Review of existing information to characterize toxic chemical mixtures commonly found at hazardous waste sites and to identify possible human health outcome relationships.
- Conduct dose–response and mechanistic studies to predict human health outcomes from exposure to toxic chemicals and mixtures.
- Evaluate adverse health outcomes in children and other susceptible populations from exposures to toxic chemicals and mixtures.
- Provide a solid basis of toxicity information for human health assessment.

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