Toxicology of Chemical Mixtures: International Perspective

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This paper reviews major activities outside the United States on human health issues related to chemical mixtures. In Europe an international study group on combination effects has been formed and has started by defining synergism and antagonism. Successful research programs in Europe include the development and application of statistically designed experiments combined with multivariate data analysis and modeling in in vitro and in vivo studies on a wide variety of chemicals such as petroleum hydrocarbons, aldehydes, food contaminants, industrial solvents, and mycotoxins. Other major activities focus on the development of safety evaluation strategies for mixtures such as the use of toxic equivalence factors or alternatives such as the question-and-answer approach, fractionation followed by recombination of the mixture in combination with a mixture design, and quantitative structure-activity relationship analysis combined with lumping analysis and physiologically based pharmacokinetic/pharmacodynamic modeling for studying complex mixtures. A scheme for hazard identification and risk assessment of complex mixtures and a consistent way to generate total volatile organic compound values for indoor air have also been developed. Examples of other activities are carcinogenicity studies on complex mixtures (petroleum middle distillates, foundry fumes, pesticides, heterocyclic amines, diesel exhaust, solid particles), neurotoxicity studies of mixtures of solvents alone or in combination with exposure to physical factors, and toxicity studies of outdoor air pollutants, focusing on particulates. Outside the United States, toxicologists and regulators clearly have a growing interest in the toxicology and risk assessment of chemical mixtures. — Environ Health Perspect 106(Suppl 6):1281–1289 (1998). http://ehpnet1.niehs.nih.gov/docs/1998/Suppl6/1281-1289feron/abstract.html

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In the United States, research programs on the toxicology of chemical mixtures have existed for several decades. These programs vary from real-life case studies (e.g., on contaminated soil or lakes, recycled drinking water, dioxins, diesel exhaust, coal tars, and chemicals released from hazardous waste sites) to the development of methods for the safety evaluation and risk assessment of both simple and complex mixtures such as new approaches for identification of genotoxic components in complex mixtures and the weight-of-evidence (WOE) approach, using interaction data in component-based risk assessment of mixtures (1–5). Moreover, in the United States, programs on more basic issues in mixture toxicology include physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling of mixtures (6–8), the use of physicochemical concepts to mechanistic elucidation of toxicologic interactions (9), the development of statistical designs for experimental studies of mixtures (10–13), and mathematical modeling of the processes involved in the carcinogenicity of mixtures of carcinogens (14). More than 10 years ago the U.S. Environmental Protection Agency (U.S. EPA) published Guidelines for the Health Risk Assessment of Chemical Mixtures (15), followed in 1990 by the Technical Support Document on Health Risk Assessment of Chemical Mixtures (16).

Outside the United States in the past, the toxicology of mixtures did not receive a great deal of attention or research efforts on mixtures were not (or were insufficiently) recognized as such. However, during the last 5 to 10 years toxicologists from academia, research institutes, and industry, as well as regulators, gradually paid more and more attention to the development of research programs on toxicology and risk assessment of chemical mixtures, recognizing that simultaneous or sequential exposure to large numbers of chemicals is a reality of potential health concern (17–34).

This paper provides a survey of major activities in Europe and Japan concerning human health issues related to chemical mixtures, using literature data and information obtained through personal inquiries. Categories of activities were identified and will be discussed individually. Obviously, possible programs or plans unknown to us could not be covered in this review.

**Terminology and Concepts in Mixture Toxicology**

Despite several attempts to provide a unified terminology for mixture toxicology (35,36), a common consistent language for this complex and challenging area of toxicology is still lacking (4). Recently, it was reemphasized that in publications on mixtures one should clearly define each term and concept used (37–39). More importantly, it has been strongly suggested that an international panel should be formed, including representatives from the U.S. EPA, the World Health Organization (WHO), and the European Community, to seek consensus on terminology (39). This panel should produce a proposal of unification to be implemented by the International Union of Pure and Applied Chemistry Toxicology Committee. Meanwhile, recommendations for study design and evaluation of combined effects of chemicals have been drafted as a first step to be discussed by the international Study Group on Combination Effects (40). This first step focuses on the

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Abbreviations used: DBF, decreased breathing frequency; MD, middle distillate; PBPK/PD, physiologically based pharmacokinetic/pharmacodynamic; PLS, projections to latent structures; PM10, current levels of ambient particulate matter air pollution; OSAR, quantitative structure-activity relationship; RIVM, National Institute of Public Health and the Environment; TEF, toxic equivalence factor; TNO, Organization for Applied Scientific Research; TVOC, total volatile organic compound; U.S. EPA, U.S. Environmental Protection Agency; VOC, volatile organic compound; WHO, World Health Organization; WOE, weight of evidence.
terms synergism and antagonism; the second step will deal with additional characterization of synergistic and antagonistic effects (40). Hopefully this study group will proceed energetically and successfully, because consensus on terminology is a prerequisite for the successful application of the framework for describing important elements of mixtures and mixture-related research as proposed by Sexton et al. (41) and for the use of the decision tree for the safety evaluation of complex mixtures as presented by Feron et al. (42). Overall, these activities as a whole indicate progress with respect to defining terms and understanding concepts in mixture toxicology.

**Designs, Statistics, and Data Analyses for Mixture Studies**

**General**

Based on analyses reported by the U.S. EPA in 1990 (16) and on her own experiences, Simmons (4) concluded that the quality of experimental designs and statistical analyses in mixture research was of significant concern: Studies on mixtures were often not well designed and statistical analysis was often poor, indicating lack of expertise and experience in designing such studies and in analyzing the results. Recently, Henschler et al. (39) reported a noticeable increase in the successful application of statistical designs such as ray designs, response–surface designs, and fractionated factorial designs in toxicologic studies of mixtures (12,43–46). It was observed that one of the advantages of these experimental designs is that the costs and the number of animals used can be greatly reduced while possible combined or interactive effects can still be detected. Moreover, it was felt that in mixture research, PBPK models may be helpful in studying possible interactions between chemicals of particular interest, recognizing that emphasis has to be put on low-dose modeling and experimental validation (38,39,47). There are several examples of successful European research programs on the development of designs and the application of appropriate statistics in mixture toxicology.

**Mixture Design**

Eide and Zahnle (48) showed the usefulness of a statistical design, multivariate data analysis, and modeling to evaluate the toxicokinetics of synthetic mixtures of n-nonane, trimethylcyclohexane, and trimethylbenzene in 12-hr inhalation experiments with rats. The total number of different mixtures studied was 21. The multivariate analysis and modeling were performed with projections to latent structures (PLS). The best models were obtained after removing all interaction terms, suggesting that there were no interactions between the hydrocarbons with respect to absorption and distribution. Uptake of n-nonane and trimethylbenzene was best described by quadratic models, whereas uptake of trimethylcyclohexane was nearly linear. All models were good, with high correlation and prediction properties, the latter after cross-validation.

**Model for Competitive Agonism**

To study sensory irritation of mixtures of formaldehyde, acrolein, and acetaldehyde as measured by decreased breathing frequency (DBF) in rats, Cassee et al. (49) used for data analysis a model for competitive agonism extended for reversible competition between the three compounds. Using both effect addition and the model for competitive agonism, the predicted DBFs were compared with the actual DBFs obtained during exposure to the mixtures of aldehydes, applying a t-test for statistical analysis. Additional multiple linear regression techniques were used to investigate whether the differences between the values calculated with the competition model and the observed values were concentration dependent. The results of the study allowed the conclusion that sensory irritation in rats exposed to mixtures of irritant aldehydes is more pronounced than that caused by each of the aldehydes separately, and that the DBF as a result of exposure to a mixture could be predicted by a model for competitive agonism, thus providing evidence that the combined effect of these aldehydes is basically a result of competition for a common receptor (the trigeminal nerve).

**Effect–Surface Designs**

The structural similarities of formaldehyde, acrolein, and crotonaldehyde indicate similar joint action of mixtures of these chemicals with respect to toxicity to the nasal epithelium. Cassee et al. (50–52) used an in vitro system (rat and human nasal epithelium cells) and a well-defined parameter for cytotoxicity (neutral red uptake) to study the combined toxicity of these aldehydes. For the studies with binary mixtures, the effects of at least 35 different mixtures were used; for the studies with mixtures of the three aldehydes, a three-level factorial design was used, resulting in 27 combinations of the three aldehydes. An isobolographic method for nonlinear concentration–effect curves and effect–surface analysis was used to evaluate the data sets of the two-compound mixtures. Data sets of the mixtures of three compounds were only subjected to effect–surface analysis. Mixtures of formaldehyde and acrolein induced less than additive cytotoxicity. Mixtures of formaldehyde and crotonaldehyde or acrolein and crotonaldehyde induced additive or slightly more than additive cytotoxic effects. Using effect–surface analysis, two-factor interactions were detected, although their contribution to the total effect of the mixtures was less than 10% and generally indicated less than additivity. Three-factor interactions were not statistically significant. Overall, mixtures of the three aldehydes did not induce marked synergistic cytotoxicity, and compared to the isobole method, effect–surface analysis proved a more rapid method to evaluate the effects of mixtures at doses ranging from no to distinct-effect levels.

To more easily recognize the additivity surface in response–surface analysis, Sühnel (46) proposed visualization of the dose–response surfaces under the assumption of additivity as a zero-interaction response surface.

**Factorial Designs**

The difficulty of studying chemical mixtures arises when one has to examine possible interactive effects of more than three compounds in a mixture. For the accurate analysis of interactive effects between chemicals in a defined mixture, statistical designs must be used to get clear and manageable experiments. Particularly useful in this respect are factorial designs; they enhance the efficiency of research because they enable economy of experimentation through the use of fractional factorial designs. Such fractionated factorial designs have been used to detect interactive effects between trace elements and the cadmium accumulation in the body (53) and to determine structure–activity relationships for halogenated aliphatic hydrocarbons (54).

Another aspect of factorial designs that deserves attention is the fact that the results are often of high precision because for every end point chosen, all data of the experiment are used to calculate a particular effect. For instance, in a recent study by Groten et al. (55), a two-level factorial design was used to describe interactive effects between nine compounds in a 4-week subacute toxicity.
study in rats. Instead of the usual comparison of e.g., five animals in the test group to five animals in the control group, the effects in this study were calculated as the difference in results between two means of 40 animals each. Therefore, main effects seen in the preliminary studies based on individual dose–response curves appeared more pronounced and more easily detectable in the factorial study.

Once the potential interactions have been detected using a fractionated design, a more accurate analysis can be performed for the particular binary mixtures to ensure and characterize the observed interactions. This stepwise approach has been successfully applied to detect mixtures of mycotoxins in contaminated food samples using the in vitro DNA synthesis inhibition bioassay in L929 fibroblasts (56, 57). Interactions between five mycotoxin species were investigated. First, a central composite design was applied to detect possible interactive effects between mycotoxins in the mixtures (27 combinations from $2^5$ possible combinations). Second, two-factor interactions of particular interest were further analyzed by means of a full factorial (5 $\times$ 5) design to characterize the nature of these interactions more precisely. It appeared that combined exposure to several classes of mycotoxins generally results in effect addition, with a few minor exceptions that indicate synergistic interactions. In general, the nature of the interactions characterized in the full factorial design were similar to those observed in the central composite design; however, the magnitude of interaction was relatively small in the full factorial design.

**Strategies for Safety Evaluation of Mixtures**

**General**

For the development of a successful strategy for safety evaluation of chemical mixtures, it is essential to comprehend the basic concepts of combined toxicologic action and interaction of chemicals (35, 58), to distinguish between whole mixture analysis (top-down approach) and component interaction analysis (bottom-up approach) (59), and to make a clear distinction between simple and complex chemical mixtures (42, 60–62).

**Toxic Equivalency Factors and the Question-and-Answer Approach**

Similar joint action (or simple joint action or dose addition) is the conceptual basis for the use of toxic equivalency factors (TEFs) and total toxic equivalency (TEQ) for a mixture of chemicals. TEFs for chlorinated dioxin congeners have been used successfully in health risk assessment of mixtures of dioxinlike chemicals (63). Highly interesting and informative studies on the toxicity and toxicokinetics of mixtures of polychlorinated biphenyls and also of chlorinated dioxins and furans have been and are still being performed at the Research Institute of Toxicology, Utrecht University, Utrecht, The Netherlands (64–69). Because the TEF concept cannot be readily transferred to other mixtures, Neumann (70) proposed a question-and-answer approach for establishing tolerance levels for mixtures of carcinogens. This approach is based on measuring hemoglobin adducts in humans as biomarkers of the most prevalent mixture components; Neumann applied this approach to mixtures of nitroarenes occurring in wastes from trinitrotoluene-based explosives (70) and plans to apply it also to diesel exhaust (71, 72). In this context it is of interest to refer to studies by Knudsen et al. (73) on genetic monitoring of bus drivers and mail carriers in the center of Copenhagen. Although the exposure levels of urban air pollution in Copenhagen have decreased during the past few years, the Knudsen et al. (73) study demonstrated a risk of genetic damage and indicated an increased cancer risk from exposure to urban air pollution, and thus provided evidence that exposure to polluted urban air still implies a health risk.

**Fractionation and Recombination of Mixtures plus Mixture Design**

Recently, a new strategy for the evaluation of complex mixtures was presented (45, 74). The new approach is based on fractionation and recombination of the mixture in combination with the use of a mixture design. The mixture studied was the organic extract of diesel exhaust particles to be examined for possible mutagenic activity. The crude extract was fractionated according to polarity into five fractions. After dissolving in dimethylsulfoxide, the three fractions containing the primary mutagens were recombined in different combinations to create new extracts. The composition of these new extracts was determined by means of a mixture design at three dose levels to support an empirical model with linear, interaction, and quadratic terms in a Taylor polynomial. The recombined extracts were tested in the Ames assay using Salmonella typhimurium strain TA 100. Multivariate data analysis was performed with PLS. The best model describing the relation between the mutagenicity and the three fractions contained two interaction terms, indicating possible synergistic or antagonistic interactions. The incorporation of dose in the mixture design and analysis of the individual data with PLS reduced the number of samples (recombined extracts) to 15, as compared to 50 that would have been necessary to determine dose–response curves of each sample (i.e., recombined extracts in different dilutions). The concept of fractionation and recombination and the use of the mixture design may in principle be extended to an unlimited number of variables. An adaptation of the mixture design to a multidimensional isobole method is currently being evaluated.

**QSAR, Lumping Analysis, and PBPK/PD Modeling**

A new ambitious approach to study the toxicology of complex mixtures has recently been published by Verhaar et al. (75), using as an example JP-5, a navy jet fuel that consists mainly of hydrocarbons. This conceptual approach integrates quantitative structure–activity relationship (QSAR) analysis, lumping analysis, and PBPK/PD modeling to predict the toxic effects of complex chemical mixtures. Unlike QSAR analysis and PBPK/PD modeling, the lumping technique is probably unknown to many toxicologists. This technique is based on grouping (lumping) chemicals with relevant similarity (for instance, chemicals with the same target organ or with similar mode of action) into pseudocomponents that are representative of the entire group, thus resulting in a considerable but scientifically justifiable simplification needed to keep such a study manageable. The details of the lumping technique have been elegantly described by Verhaar et al. (75).

**Top Ten Chemicals and Weight-of-Evidence Approaches**

Feron et al. (60, 61) developed a two-step procedure for the safety evaluation of certain complex mixtures, namely, selection of the top 10 chemicals i.e., the 10 most risky chemicals in a mixture (step one), followed by hazard identification and risk assessment of the 10 selected chemicals to be approached as a simple mixture (step two). The second step of this procedure is essentially similar to the WOE scheme for assessment of combined toxicologic actions or toxicologic interactions between
chemicals, which was developed by Mumtaz and Durkin (3) and applied for risk assessment purposes to mixtures of selected chemicals released from hazardous waste sites (76,77). Although suffering from a number of drawbacks, the WOE scheme should be considered one of the most universal approaches to estimate possible interactions between compounds in a given mixture. However, the method has not been sufficiently validated using experimental or epidemiologic data. Therefore, in a joint effort, the U.S. Agency for Toxic Substances and Disease Registry (ATSDR) and the Organization for Applied Scientific Research (TNO) in The Netherlands are currently in the process of examining whether the general WOE approach can be used to account for interactions between chemicals observed in experimental animals. To this end, the huge amount of data obtained in several mixture studies in rats carried out by TNO during the past few years is being used. Preliminary results indicate that for the mixtures of similar-acting chemicals, the WOE method quantitatively predicts the observed combined actions. For the mixtures of dissimilar-acting chemicals, the quantitative evaluation seems less accurate (78,79). In the next few years the WOE approach will be under continuous review by the ATSDR, the TNO, the U.S. EPA, and the Netherlands Ministry of Public Health, Spatial Planning and the Environment, focusing on improving the quantitative evaluation and target-organ toxicity.

Scheme for the Safety Evaluation of Complex Mixtures

As mentioned previously, a prerequisite for successful toxicologic evaluation of chemical mixtures is to clearly distinguish between simple and complex mixtures as defined by Feron et al. (42,60,61), as well as between the top-down and bottom-up approach (42). A genuine bottom-up approach is virtually impossible for complex mixtures, although the aforementioned approach suggested by Verhaar et al. (75) is a pseudo bottom-up approach using lumping analysis to keep the chemicals to be dealt with to manageable numbers. For some complex mixtures such as welding fumes or diesel exhaust, which are available for testing and for which the chemical composition is fairly well characterized, the top-down approach might be appropriate. For others, e.g., the atmosphere at a hazardous waste site or in a workplace, whole mixture analysis seems impracticable. According to a working group on complex mixtures (62), the usefulness of a certain approach depends on the context in which one is confronted with the complex mixture, and on the amount, type, and quality of the information available on the chemistry and the toxicity of the mixture. This working group also recommended the development of a decision tree for the safety evaluation of complex mixtures (62). Challenged by this recommendation, we developed a scheme for hazard identification and risk assessment of complex mixtures (42). After dividing mixtures into simple and complex mixtures, the scheme continues with a dichotomy of complex mixtures into mixtures readily available for testing in their entirety and mixtures virtually unavailable for testing as a whole. Examples of the former are drinking water, diesel exhaust, welding fumes, tobacco smoke, and new food products; examples of the latter are workplace atmospheres, coke oven emissions, and atmospheres at waste sites. Other conspicuous elements of the scheme are the inclusion in the scheme of the top 10 and pseudo top 10 approaches (in essence, selection of the e.g., 10 most risky chemicals or pseudochemicals to be dealt with as a simple mixture) for complex mixtures that are readily available for testing in their entirety. The aim of the scheme is to stimulate progress with hazard identification and risk assessment of complex mixtures, bringing together and effectively using all available relevant information, methods, technologies, expertise, and experience (42).

Total Volatile Organic Compounds Value

Indoor air samples may typically contain 100 or more volatile organic compounds (VOCs) at concentrations in the range of 1 to 100 μg/m³ (80). For relatively few of them, exposure limits have been established, and little or no attention has been paid to assessing health and well-being effects from simultaneous exposure to VOCs. There is, however, a pressing need for adequate methods to assess the health and well-being consequences of simultaneous exposure to air pollutants, particularly because of the general public's increasing concern about indoor air quality. A possible approach is the use of the total volatile organic compound (TVOC) concentration, an entity meant to characterize the pollutant load in terms of VOCs in the study of health and comfort complaints. Different procedures are being applied to generate TVOC values; thus, reported values are often not comparable. The urgent need for a widely accepted approach for generating TVOC values and for how to use them for health risk evaluations has prompted a European Collaborative Action on Indoor Air Quality and Its Impact on Man (81) to establish a working group to develop guidance on this topic. Meanwhile, this working group (Working Group 13) has produced the final report (81). The report suggests a definition of TVOCs referring to a specified range of VOCs, and it proposes a method for the measurement of this TVOC entity. The measured concentrations of identified VOCs within the specified range are summed up. Concentrations of nonidentified compounds in toluene equivalents are added, and together with the identified VOCs they give the TVOC value. On one hand, the report recognizes that in view of the fact that there are few controlled human exposure studies and the results are not confirmed and that the results of epidemiologic studies are inconsistent, it is currently not possible to conclude that sensory irritation is associated with the sum of mass concentrations of VOCs at the low exposure levels typically encountered in nonindustrial indoor air. Therefore, although the likelihood of sensory effects will increase with increasing TVOC concentrations, at present no precise guidance can be given on which levels of TVOCs are of concern from a health and comfort point of view, and the magnitude of protection margins cannot be estimated. On the other hand, the general need for improved source control to diminish the pollution load on indoor environments from health, comfort, energy efficiency, and sustainability points of view leads to the recommendation that VOC levels in indoor air should be kept as low as reasonably achievable (81).

Carcinogenicity Testing of Mixtures

There are numerous complex chemical mixtures that may affect or are known to affect cancer development in humans. In this paper we discuss a few examples of recent conspicuous carcinogenicity studies in experimental animals. During the XXXVI European Congress of Toxicology, Arhus, 25–28 June 1997, Prizzen et al. (82) reported the final results of a 10-year research program on the effects of irritation (cytotoxicity followed by regenerative hyperplasia) on mouse skin carcinogenicity of petroleum
middle distillates (MD). MD are petroleum-derived hydrocarbons boiling between 175 and 370°C. A 2-year mouse skin-painting study evaluated whether tumor development was a secondary response to skin irritation by comparing the effects of equal weekly doses of irritating (undiluted) and nonirritating (diluted in mineral oil) MD. Straight-run kerosene, straight-run gas oils, and catalytically cracked light cycle oil were tested. All produced severe skin irritation and increased skin tumor response when tested undiluted. When diluted, irritant effects of kerosene and straight-run gas oils containing low levels of carcinogenic polycyclic aromatic hydrocarbons were reduced, and no significant increase in the number of skin tumors was observed. However, catalytically cracked light cycle oil containing relatively high levels of carcinogenic polycyclic aromatic hydrocarbons significantly increased skin tumor frequency even when skin irritation was low. These data indicate that straight-run MD containing low levels of carcinogenic polycyclic aromatic hydrocarbons do not produce skin tumors in mice as long as prolonged skin irritation is avoided. Thus, as long as sustained skin irritation is avoided, such MD should not present a skin cancer hazard to humans.

Foundry fume is a complex mixture of gases and fine particles generated during the casting process when molten metal is poured into sand molds bound together with organic binders. Humfrey et al. (83) studied the potential carcinogenicity of three different fumes in a 2-year rat study using an intrabronchial pellet implantation technique. The fumes were tested concurrently in in vitro assays for mutagenicity, unscheduled DNA synthesis, free radical DNA damage, and micronucleus induction. The rat bioassay failed to demonstrate a carcinogenic response, although an increase in preneoplastic changes (squamous metaplasia and dysplasia of the bronchial epithelium) was seen in all fume-treated groups. The fumes were positive in many in vitro assays and the degree of activity correlated with the polycyclic aromatic hydrocarbon content of the fumes. It was concluded that the use of a battery of in vitro assays for different genotoxic end points provides information useful for the overall assessment of carcinogenicity of complex mixtures such as foundry fumes.

Many impressive in vivo studies on the potential carcinogenicity or possible modifying effects of mixtures of highly important industrial chemicals or food components have been carried out at the Nagoya City University, Nagoya, Japan, as exemplified by recently published studies on pesticides by Ito et al. (84,85) and on heterocyclic amines by Hasegawa et al. (86,87). A characteristic of these Japanese studies is scientific thoroughness combined with a pragmatic approach, which yields very useful results.

Another impressive program on long-term carcinogenicity testing of different types of (complex) mixtures in different animal species has run for more than a decade at the Fraunhofer Institute of Toxicology and Aerosol Research in Hannover, Germany. For examples of papers concerning this research program, we refer to review papers by Muhle et al. (88) and Heinrich (89).

Neurotoxicity of Mixtures of Solvents and Physical Factors

The so-called solvent syndrome has attracted a great deal of attention in Europe. Seeber et al. (90) compared a number of methods to evaluate the neurotoxic potency of mixtures of solvents and concluded that cumulative lifetime exposure and lifetime weighted-average exposure are the best exposure indices to be used to predict neurobehavioral dose-response relations for mixtures of solvents, whereas liters of solvents used per day years turned out to be the most reliable exposure index for predicting neurobehavioral deficits. An ambitious and interesting research program focusing on the effects of combined exposure to solvents and physical factors such as noise and light is in progress at the National Institute for Working Life in Solna, Sweden, in cooperation with other institutes inside and outside Sweden (91,92). Other activities in this area are improvement of the methods for risk assessment of hearing loss (93), neurobehavioral studies among paint formulators exposed to low levels of mixtures of solvents (94), studies on peripheral nerve impairment among workers exposed to mixtures of hexane and toluene (95), and toxicokinetic studies in humans exposed to n-hexane and methyl ethyl ketone (96).

Endocrine Disruptors

In Europe a range of scientific reports on endocrine disruptors has been published recently (97–100). There is much debate in the field regarding possible combined or interactive effects of chemicals in mixtures of endocrine disruptors. A research project currently underway at the Institute for Environment and Health, University of Leicester, Leicester, United Kingdom, deals with validation of in vitro systems used so far, and includes studies on possible interactions between endocrine disruptors, both natural substances and synthetic existing chemicals (101).

Outdoor Air Pollution Particulate and Gaseous Pollutants

The growing body of epidemiologic data suggests that current levels of ambient particulate air pollution (PM10) and ozone are positively and significantly associated with adverse health effects. For instance, worsening of asthma appears to be associated with acute exposure to relatively low mass concentrations of the particles. Air quality data show that PM10 has a typical bimodal or even trimodal distribution with a fine mode and an ultrafine mode, and that particles consist of a complex mixture of primary (carbonaceous) and secondary (acid and partly neutralized inorganic) aerosols. A limited number of experimental studies has suggested that combined exposure to solid particles or liquid aerosols and gases or vapors such as O3, SO2, HNO3, and aldehydes causes increased airway effects as compared to the effects of the individual substances (102–108). There is at present no biologically plausible explanation for the adverse effects associated with exposure to PM10 that would relate these effects with the mass, number, size, chemical, and physical composition, or the origin of PM10. One of the research initiatives of the National Institute of Public Health and the Environment (RIVM) of The Netherlands is to investigate the joint action of ambient fine particles and gases. Laboratory experiments using model aerosols such as sulfates, nitrates, and carbon black, in combination with ozone and/or nitrogen dioxide, will be performed in several animal species. Moreover, inhalation toxicity studies using a virtual impactor system to concentrate real-world particles (109) at different locations will also be carried out. For this purpose, the RIVM has constructed a mobile laboratory that can be placed at locations with different air quality such as urban, rural, highly industrialized areas, or locations that are dominated by heavy traffic. It will also be possible to remove or add gases to the concentrated PM10 for studying the potential interactive effects of PM10 and gases.
Emissions from Waste Incinerators
The emissions from waste incinerators represent a low-level mixed exposure. The Institute for Environment and Health, University of Leicester, has reviewed the health effects on an individual chemical basis but also assessed the epidemiologic evidence for adverse health effects in populations surrounding these incinerators—populations presumably exposed to the mixture of emitted chemicals. There was no evidence of an increased risk of disease in populations living close to waste incinerators (110).

Concluding Remarks
Somewhat to our surprise, the present survey on major activities outside the United States (mainly in Europe) concerning human health issues related to chemical mixtures reveals a large number of programs and research groups involved in the toxicology and risk assessment of chemical mixtures. Moreover, this survey was far from exhaustive, and thus most likely incomplete. The activities vary from better defining basic concepts and developing designs for toxicity studies of simple and complex mixtures, to the use of pragmatic approaches or straightforward methods to examine the toxicity of real-life complex mixtures. This great variety is not surprising because chemicals, and thus also mixtures of chemicals, are everywhere, and we are confronted with their potential adverse effects in living organisms all the time. These experiences and considerations call for international conferences, symposia, and workshops on the toxicology and risk assessment of mixtures, and also for more international cooperation, e.g., through exchange of visiting scientists and joint research projects. The formation of specialty sections on mixture toxicology in national and international societies of toxicology might be a way to identify priorities and stimulate cooperation in this challenging area of toxicology.

During an opening speech for a meeting on WHO Air Quality Guidelines for Europe, Bilthoven, 28–31 October 1996, a representative of the European Commission designated research on mixtures of major outdoor air pollutants as one of the two top priorities she had identified for the next few years (111). Clearly, toxicologists and regulators outside the United States also have a growing interest in the toxicology and risk assessment of chemical mixtures. Another positive development is the increasing attention that industry is directing to the toxicology of mixtures, with a gradual understanding that this branch of toxicology is not a threat to industry because it becomes clear that as a rule exposure to mixtures of chemicals at low nontoxic doses of individual chemicals is of no health concern, but at the same time realizing that occasionally a mixture may present a real health concern. Moreover, there is an increasing awareness in the general public about simultaneous exposure to compounds such as food additives and contaminants, outdoor air pollutants, pesticides, medicines, and hazardous substances in the workplace. For occasions of real but also of perceived health concern, tools for hazard identification and risk assessment of simple and complex chemical mixtures must be available when they are needed.

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