# International Issues on Human Health Effects of Exposure to Chemical Mixtures

**Victor J. Feron,[1] Flemming R. Cassee,[2] John P. Groten,[1] Petronella W. van Vliet,[3] and Job A. van Zorge[4]**

[1]TNO-Nutrition and Food Research, Toxicology Division, Zeist, the Netherlands; [2]National Institute of Public Health and the Environment, Laboratory for Health Effects Research, Bilthoven, the Netherlands; [3]Health Council of the Netherlands, The Hague, the

In this article, we highlight new developments and recent studies concerning adverse human health effects related to chemical mixtures. One group of activities comprises the development of a new computer program for analyzing mixture studies and a mathematical model as a basis for combination rules that predict the toxicity of mixtures. Other new activities in the area of experimental studies are the application of gene expression technologies in mixture research, and pattern recognition as a tool in safety evaluation of complex mixtures. A "bottom-up" approach for chemosensory detection of mixtures has recently been presented. Other topics include a method for the safety evaluation of natural flavoring complexes, and an evaluation of the possible health effects of the simultaneous intake of food additives. Examples of issues related to mixtures of airborne chemicals are potential interaction of fine particles and gaseous pollutants in ambient air, nasal cancer associated with inhaled chemical mixtures, and the recommendation of a limit value for volatile organic compounds. Topics of a more strategic nature include studies concerning the public health effects of large airports, and the development of criteria for a harmonized classification of chemical mixtures. This overview illustrates that strategies to tackle the safety evaluation of combined exposures and complex mixtures as well as models facilitating the interpretation of findings in the context of risk assessment of mixtures have become increasingly important. It is true that exposure of humans to chemical mixtures is the rule rather than the exception, and therefore health risk assessments should focus on mixtures and not on single chemicals. It is also true, however, that humans have learned to cope with exposure to huge numbers of chemicals simultaneously (food, water, air, soil, and consumer products). Therefore, in view of limited resources for toxicological research, the focus in toxicology should be on priority mixtures—priority being determined by (estimated) health risk (= toxicity and exposure). *Key words:* airports, classification, flavors, irritants, mathematics, mixtures, models, statistics, toxicogenomics, toxicology. *Environ Health Perspect* 110(suppl 6):893–899 (2002).

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In 1998 we reviewed the major activities in Europe and Japan on health issues related to chemical mixtures (*1*). Although this survey was not exhaustive and thus most probably incomplete, we were surprised by the large number of research groups involved in the toxicology and risk assessment of mixtures. The research programs varied from defining basic concepts in mixture toxicology to straightforward toxicity and carcinogenicity testing of complex chemical mixtures. The survey also revealed a growing interest among toxicologists and regulators in the toxicology and risk assessment of chemical mixtures, with the realization that simultaneous or sequential exposure to large numbers of chemicals is a potential health concern. In this respect it is interesting to note that already in 1985 the Health Council of the Netherlands included in one of its advisory reports to the Dutch government a chapter on the potential health consequences of combined exposure to chemicals, formulating some basic rules on how to deal with combined exposures in standard setting (*2*). Methods for dose addition and response addition were recommended.

In the United States, research programs on the toxicology of chemical mixtures have existed for decades, and more than 15 years ago the U.S. Environmental Protection Agency (U.S. EPA) published Guidelines for the Health Risk Assessment of Chemical Mixtures (*3*), followed some years later by the Technical Support Document on Health Risk Assessment of Chemical Mixtures (*4*). The research programs varied widely and included studies on basic issues in mixture toxicology such as physiologically based pharmacokinetic/pharmacodynamic modeling of mixtures, use of physicochemical concepts for elucidating toxicological interactions, development of statistical designs for experimental studies of mixtures, and mathematical modeling of the processes involved in the carcinogenicity of mixtures of carcinogens (*5–8*). Moreover, thousands of mutagenicity studies and tens of carcinogenicity studies have been performed on (fractions of) real-world mixtures such as diesel engine emissions, recycled drinking water, urban air samples, tobacco smoke, foundry fumes, and incinerator emissions (*9–33*).

The passage of the Food Quality Protection Act and the Safe Drinking Water Act Amendments in 1996 (*34*) has further raised awareness of chemical mixtures health issues and has resulted in research on health risks associated with multiple pathways of exposure and chemical mixtures. In the coming years, the U.S. EPA and the Agency for Toxic Substances and Disease Registry are expected to release further guidance on chemical mixtures risk assessments (*34*).

In this article, an update of our previous one (*1*), we review recent studies and new developments concerning the toxicology and risk assessment of chemical mixtures. Activities were grouped and will be discussed groupwise.

## Conceptual Issues

*CombiTool—a new computer program.* CombiTool is a new computer program for the analysis of the toxic effects of mixtures (*35*). It performs model calculations and analyses of experimental combination effects for two or three chemicals according to both Bliss independence (response addition) and Loewe additivity (dose addition) criteria. These data can be displayed as a difference response surface. They can be used as a general analysis of the difference between Loewe additivity and Bliss independence. Zero interaction response surfaces are calculated from single-chemical dose–response relationships and compared with combination data obtained experimentally. CombiTool has a graphics facility that allows direct visualization of the response surfaces or the corresponding contour plots and the experimental data. As far as the authors know (*35*), it is the only computer program that offers the possibility of analyzing mixture studies according to Loewe additivity and Bliss independence criteria. Earlier versions of CombiTool have already been used successfully in several studies on combined-action assessment (*36–39*).

*Mathematical basis for combination rules.* A mathematical model is being developed as a

basis for combination rules that predict the effects of chemical mixtures, starting from the concentrations of the individual chemicals in the mixtures (*40*). This representational model begins with empirical ordering of different mixtures of the same chemicals with respect to a relevant adverse effect of the components in the mixture. This approach is taken from measurement theory (*41–43*). Qualitative properties of the ordering are postulated, then the combination rule that predicts this effect ordering is mathematically derived. Different sets of properties imply different combination rules. The valid set of properties for a certain mixture is found by empirically testing whether the ordering satisfies the critical properties. An important property is independence, which in the present approach is formulated in terms of the effect ordering. This new approach is particularly useful if the insight in the mechanism of toxicity of the individual chemicals is not sufficient for predicting the effect of their mixture. It is meant as an alternative for the use of combination rules based on classifications of the combined action with classes such as independent similar action, independent dissimilar action, and interaction. The representational approach is more rigorous and bases the selection of a combination rule on testable postulates. A model consisting of four postulates concerning the effect ordering of combined chemicals serves as an example (*40*).

## Experimental Studies and Upcoming Technologies

*Interaction of particles and gases in ambient air.* Real-world exposures to air pollutants are rarely to single pollutants but rather are a mixture that reflects the integration of many sources, emission constituents, or ongoing photochemical processes in the atmosphere. Apart from a range of gases, the atmosphere also contains particulate matter (PM)—a mixture of solid particles and liquid droplets that may vary in mass, size, and chemical composition, depending on the sources and the meteorological conditions. Many components may be adsorbed to the solid particles, for example, acids, partly neutralized salts, aliphatic and (polycyclic) aromatic organic compounds, metals, and biomaterial (allergens, pollen fragments, and endotoxins). PM levels relevant to human health effects are commonly expressed on the basis of the mass concentration of inhalable particles, defined to contain particles with an aerodynamic diameter ≤10 µm because only these particles can penetrate into the airways and lungs. There is still debate about the validity of this type of metric; the number, surface area, or specific chemicals might be a more relevant measure for setting standards. Although air quality data have revealed that ambient

aerosols have typical trimodal mass or number distributions, showing peaks at coarse mode (2.5–10 µm), fine mode (0.1–2.5 µm), and ultrafine mode (<0.1 µm, dominated by the number of particles) particles (*44*), PM is considered a complex mixture because of the large number of chemical constituents in each of these classes.

Inhalation studies addressing the health effects of PM use either model compounds predominantly present in the complex mixtures or the mixture itself, applying the technology of concentrators (*45*). At present the prime goal of inhalation studies is to confirm epidemiological associations between PM levels and observed morbidity and mortality by showing a causal relationship. The identification of the responsible component(s) is the secondary goal.

Synergistic interactions of pollutant gases and ambient PM have been studied from the early 1950s. Amdur et al. (*46*) established that fossil fuel irritants such as $SO_2$ can interact physicochemically with soluble metal salts to generate particles intrinsically more toxic than the primary compounds. More recently, $SO_2$ was shown to react with combustion-associated ZnO emission PM in a humidified atmosphere, resulting in acid sulfate that can be carried deep into the lungs of test animals (*47,48*). Other experimental studies have supported the potential for combined gas-particle interactions such as fine carbon or diesel, acidic, or dispersed ambient particles combined with (in)organic gases or vapors (e.g., $O_3$, $NO_2$, $SO_2$, $HNO_3$, aldehydes) (*49–51*). The results of these studies suggest that particles can act as reactants or carriers to deliver toxicants to the deep lung. A restricted number of human studies have been conducted similar to Amdur's $SO_2$ interaction experiments (*52–54*), using acids and oxidants, but little evidence of synergism between PM and gases has been observed, perhaps because of the small numbers of subjects tested in most studies. Recently, in a 4-week inhalation study with 0.5-µm particles combined with exposure to carbon black (50–100 µg/m$^3$), ammonium bisulfate (70 µg/m$^3$), and ozone (0.15 ppm or 300 µg/m$^3$), the combination showed more deleterious effects than the components alone (*55,56*). The effects included decreased alveolar macrophage function and increased lung collagen concentration and lung cell turnover rates, although there were no indications for increased lung permeability and inflammation. Acute inhalation to ultrafine and fine carbon black in combination with ammonium nitrate in healthy and compromised rats, however, did not reveal an interactive toxicological response (*57*).

***"Bottom-up" approach in chemosensory detection of mixtures.*** The topic of chemosensory responses to mixtures has

relevance not only to the basic understanding of chemosenses but also to a variety of applied topics such as food flavors and air quality (*58,59*). Many studies addressing the human perception of chemical mixtures focus on olfaction and suprathreshold concentrations, expressing results in terms of the response (*59,60*). Fewer studies focus on threshold exposure levels and express the results in terms of the stimulus (that is, concentration of the chemical) (*59,61*). Using binary mixtures of 1-butanol and 2-heptanone, Cometto-Muñiz et al. (*59*) developed a strategy for testing chemosensory detectability of mixtures by measuring detectability functions for odor, nasal pungency, and eye irritation in normosmics and anosmics. The results provide support for the existence of dose additivity in the detection of chemical mixtures at perithreshold exposure levels. This appeared to hold for all three end points (odor, nasal pungency, eye irritation). The data for odor suggested that perithreshold stimulation might elicit little or no mutual inhibition between components in the mixture. At levels progressively above the threshold, inhibitory interaction (competitive agonism) appeared to grow. At very low concentrations of an odorant mixture, there might be negligible competition between components for binding to receptors (*59*). As the concentration of the odorant in the mixture increases, the competition for binding to the olfactory receptors also increases. The structural similarity between odorants in a mixture, and thus their ability to bind to a smaller or larger overlapping family of receptors, then becomes a crucial factor for understanding the type of combined effects of the chemicals in the mixture (*59*). In this respect it is of interest to refer to the observation of Cassee et al. (*62*) that nasal sensory irritation in rats exposed to a mixture of formaldehyde, acrolein, and acetaldehyde 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 trigeminal nerve receptor.

Only a systematic study of a number of binary mixtures in which the components differ in structure from one another to various degrees can answer the question of whether an increasing degree of molecular difference between components will reduce the competition for the same receptor and thus also the degree of competitive agonism in mixtures to produce odor detection. This issue will be addressed in future studies (*59*).

The above approach of studying chemosensory detection of mixtures compared with detection of the separate components via measuring complete detectability functions (that is, measuring functions for

both odor, nasal pungency, and eye irritation) is a classic bottom-up approach, which indeed might take much time and effort before providing data relevant to real-life mixtures consisting of dozens of chemicals. Nevertheless, it is worth the cost and effort because this approach may lead to a better understanding of the chemosensory impact of certain mixtures such as volatile organic compounds (VOCs) (59).

*Pattern recognition in safety evaluation of complex mixtures.* In case of complex mixtures that have not been properly tested toxicologically and that may contain large numbers of unidentified components, pattern recognition techniques may be used. Principal component analysis (PCA) is used to detail the chemical characteristics of a mixture by comparing it with other mixtures, either as fingerprints or as detailed information on identity and quantity of each component (63). This kind of comparison of composition patterns requires a database with compositional information obtained in a standardized way. PCA has been used to analyze gene expression data from DNA arrays (64). PCA appeared to be able to identify broad patterns of expression alterations. Using PCA, genes could be clustered into related expression patterns (65).

A more sophisticated use of pattern recognition is to directly derive from existing toxicity data on certain mixtures imaginary toxicity data for the complex mixture of concern. This requires the use of multivariate regression techniques, for example, projections to latent structures (PLS) (63). With PLS the toxicity of a complex mixture can be predicted using the toxicity and the physicochemical data of other complex mixtures along with the physicochemical data of the mixture of concern. An advantage of this approach is that there is no need to explicitly identify a complex chemical mixture that is chemically very similar to the mixture of concern.

Overall, in the hands of experts, pattern recognition techniques are considered powerful tools for the safety evaluation of complex chemical mixtures.

*Toxicogenomics in mixture research.* Advances in gene expression technology provide the means to profile expression of thousands of messenger RNAs simultaneously, and similarly, the expression of proteins within a cell (66–68). Toxicology will benefit enormously from the application of genomics (transcriptomics, proteomics, metabolomics) to analyze chemically induced alterations in gene expression. Because of the integrated and holistic nature of genomics, in all likelihood such changes in gene expression can be identified at exposure levels lower than those affecting more conventional parameters (34,69). Once validated, the use

of combined transcriptomics, proteomics, and metabolomics will make it possible to map early toxicity-related alterations in cells, tissues, or animals exposed to chemicals, and thus will lead to insight in numerous toxicologically relevant cellular processes simultaneously. Clearly, validation studies are crucial, and indeed, some have been performed (66,70,71). However, many more will have to follow to understand the strengths and limitations of this new very promising technology (72).

The use of gene expression technologies such as microarrays (gene/DNA chips) is most suitable to detect joint or interactive effects of chemical mixtures. As part of a program on the validation of the weight of evidence (WOE) methodology for assessing hazard and risk of defined mixtures, an oral subacute study in rats that examines the toxicity of lead, mercury, benzene, and trichloroethylene alone or as a mixture is under way (73). In addition to conventional toxicity parameters, changes in messenger RNAs and protein expression levels are being measured in various target tissues.

## Strategic and Regulatory Issues

*Drinking water disinfection byproducts.* Drinking water treated with chemical disinfectants such as chlorine and ozone contains disinfection byproducts (DBPs) formed as a consequence of the reaction between the disinfectants and natural organic matter present in the source water (74). Toxicological and epidemiological studies indicate DBPs may cause adverse effects in humans (10,75). To properly assess the toxicity and potential adverse health effects of DBPs, researchers widely recognize that drinking water (treated with disinfectants) should be approached as a variable, complex, very diluted chemical mixture (75–77) with the following main characteristics: *a*) large numbers of chemicals occurring at very low levels, *b*) a large fraction (about 50%) of unidentified DBPs, and *c*) lifetime exposure of the consumer. Different complex mixtures require different approaches to evaluate their safety, and the usefulness of a certain approach depends on the context in which one is confronted with the mixture and also on the amount, type, and quality of the available data on the chemistry and toxicity of the mixture. Mixtures may be virtually unavailable or readily available for testing in their entirety. A possible approach is to focus on the most risky chemicals (10, for example) in a mixture, assuming the risk of the entire mixture is largely determined by the risk of the mixture of these selected components (77,78).

The safety evaluation of DBPs in drinking water is extremely complex. It has, nevertheless, a very high priority because of

the huge exposure in terms of exposure time and number of individuals exposed. As a consequence, there is a tendency to develop all-embracing research programs using every methodological tool available (79,80). We feel this is an unrealistic and thus little helpful strategy. We agree with Groten (77) that prioritization of the various groups of DBPs is badly needed, focusing on an approach that considers the large fraction of unidentified DBPs.

*Public health effects of large airports.* Recently, the Health Council of the Netherlands published two reports addressing the topic of large airports and public health (81,82). Quality of life was included in the definition of public health. The key question was "Do large-airport operations affect public health?" The answer was "yes." Major factors were air pollution, noise, and safety, with aircraft noise generally being considered to have the most significant impact on people living in the vicinity of a large airport (81,83). Clearly, environmental factors other than chemical agents are of major significance.

Air pollution around large airports is comparable to that in urban areas, and thus the health consequences are also similar viz. increased mortality, decreased life expectancy, increased cardiovascular and respiratory tract complaints, and odor annoyance. Noise caused by large airport operations may lead to hypertension, ischemic heart disease, decreased study performance, sleep disturbance, and general annoyance (disgust, anger, dissatisfaction, resentment, discomfort) (84,85). Fear and anxiety about aircraft crashes impact on quality of life and contribute to stress. Other factors with possible impact on public health are soil and water pollution, import of infectious diseases (malaria, for instance), landscape appearance, and perception of risk and external safety.

The report ended with an attempt to integrate the findings. Is the impact of cumulative exposures the sum of the effects of the separate exposures, or is the cumulative exposure characterized by supra-additivity (or infra-additivity)? This question could not be answered. Studies addressing the question were not available in the scientific literature. Under the supervision of the National Institute of Public Health and the Environment in the Netherlands, such a study is currently being performed (86).

*Safety evaluation of natural flavoring complexes.* Natural flavoring complexes are mixtures of constituents obtained by applying physical separation methods to botanical sources. Sources include pulp, bark, peel, leaf, and flower of fruits, vegetables, spices, and other plants. Many of the approximately 300 natural flavor complexes have a food origin, for example, lemon, basil, and celery seed oils.

The method for the safety evaluation of natural flavoring complexes (the naturals paradigm) is intended only for the safety evaluation of natural flavor complexes derived from higher plants to be used as flavoring substances for food and beverages. The naturals paradigm is a procedure that begins with a review of available data on the history of dietary use of the natural complex, then prioritizes constituents according to their relative intake (from use of the natural complex as a flavoring substance) and their chemical structure (*87*). The method further uses the concept of threshold of toxicological concern (*88*) and assigns constituents to one of three structural classes (*89–91*). Another aspect of the naturals paradigm involves the evaluation of constituents of unknown chemical structure. As a conservative default assumption, the total intake of all unknowns is considered together and placed in the structural class of greatest toxic potential and thus compared with the most conservative exposure threshold. The paradigm also addresses the concept of joint action among structurally related constituents. If a common pathway of intoxication has been identified or can be reasonably predicted on the basis of structure–activity relationships for a group of constituents, the combined intake of those substances will be compared with the appropriate human exposure threshold of concern. Ultimately, the procedure focuses on those constituents or groups of constituents that, because of their intake and structure, may pose significant risk from consumption of the natural complex. With the developed strategy, the overall objective of the naturals paradigm can be attained—that no reasonably significant risk associated with the intake of natural complexes will go unevaluated. A publication describing in detail the different steps of the naturals paradigm and containing example evaluations is in progress (*87*).

*Combined intake of food additives.* Food additives are authorized in the European Union (EU) on the basis that they constitute no health risk to the consumer at the proposed level of use. Although additives at their permitted use levels are considered safe, there are concerns that simultaneous intake of different additives could be of potential health significance. Therefore, the International Life Sciences Institute Europe Acceptable Daily Intake Task Force established an expert group of independent scientists to analyze the possibility of health implications of joint actions and interactions between the 350 food additives currently approved in the EU (*92*). All approved additives allocated a numerical acceptable daily intake value were studied. Target organs were identified on the basis of the effects reported at doses above the no-observed-adverse-effect levels in animal or human studies. Descriptions of the pathological and other changes reported were used to assess whether different additives sharing the same target organ would produce a common toxic effect. In all but a very few cases, the possibility of joint actions or interactions could be excluded on scientific grounds. The exceptions were some additives with effects on the liver (curcumin, thiabendazole, propyl gallate, and butyl hydroxy toluene), the kidneys (diphenyl, *o*-phenylphenol, and ferrocyanide salts), the blood (azorubine and propyl gallate), and the thyroid (erythrosine, thiabendazole, nitrate). In-depth consideration of both the specific use and the intake levels of these last-mentioned additives led to the conclusion that joint actions or interactions among these additives are a theoretical rather than a practical concern.

When approving future additives that show target organ toxicity, investigators should consider the possible joint actions or interactions of previously approved additives on the basis of a common mechanism of toxicity (*92*).

*Nasal cancer associated with inhaled chemical mixtures.* Nasal cancer occurs in experimental animals after chronic exposure to a wide range of inhaled chemicals (*93,94*). Although exposure to several of these chemicals is common in industrial and domestic environments, epidemiological studies have not provided convincing evidence that exposure to the individual chemicals is associated with nasal cancer. The reverse seems to be true for inhalation of chemical mixtures. The evidence for nasal carcinogenicity of inhaled mixtures in experimental animals is very limited, whereas there is ample evidence that occupational exposure to certain chemical mixtures is associated with increased risk of nasal cancer (*94*). Examples of such carcinogenic (complex) chemical mixtures are wood dust, textile dust, chromium-containing materials, and leather dust. Whether wood-preserving agents contribute to the effects of wood dust on the sinonasal mucosa has not yet been determined (*95*). Effects may also be gender specific. A recent analysis revealed that nasal adenocarcinomas due to wood-dust exposure are associated with a higher risk in men but not in women, whereas exposure to leather dust is associated with an excess in both genders (*96*). Moreover, tobacco smoking should not be overlooked as a risk factor for sinonasal cancer, causing mainly squamous cell carcinomas (*96,97*). On the other hand, a recent survey carried out in the United States has thrown doubt on the significance of wood dust as a human carcinogen. Among men who reported exposure to wood dust, there was an elevated risk of total mortality but no excess of sinonasal cancer (*98*).

It is remarkable that these carcinogenic mixtures are aerosols, suggesting that their particulate nature may be a factor in their potential to induce nasal cancer in humans (*94*). Cigarette smoke as a complex mixture seems to be an exception, as it was found to induce inflammation, degeneration of olfactory epithelium, and hyper- and metaplasia of the nasal respiratory epithelium in experimental animals (*99–103*). However, in all likelihood these nasal effects are caused by vapor phase components such as formaldehyde, acetaldehyde, acrolein, and furfural, and not by the particulate phase of cigarette smoke.

Whatever the identity of the responsible cigarette smoke components, these findings in experimental animals correlate with the excess risk of sinonasal squamous cell carcinoma observed in smokers in Europe (*96*). In this respect it is also relevant to emphasize recent findings by Klein et al. (*104*) that reveal 100% incidence of nasal tumors in rats after long-term exposure by inhalation to 2.4 ppm 1-nitroso-4-methylpiperazine, resulting in a total dose of 86 mg/rat. This was almost two orders of magnitude lower than the dose inducing nasal tumors in rats after oral administration of this nitroso compound. These findings suggest a major role for carcinogenic nitrosamines in tobacco smoke upper and lower respiratory tract carcinogenesis.

*Volatile organic compounds from building materials.* To evaluate and regulate emissions of VOCs from building materials, Nielsen et al. (*105*) suggested the use of indoor air standards or guidelines, or when these are not available, occupational exposure limits (OELs) divided by a default safety factor of 40 or another factor when justifiable. A committee of the Health Council of the Netherlands (*106*) considered the predictive value of OELs for assessing the potential health effects of emissions from building materials too low to justify their use for this purpose. According to this committee, the exposure period (8 hr/day; 5 days/week; 40 years) and the target population (workers) differ too much from the indoor environment situation. This committee also discussed the use of air quality guidelines developed by the World Health Organization for outdoor air (*107*) but advised against their use for a practical reason: such guidelines have been established for only a few VOCs. The committee recommended the use of the chemosensory effect of VOCs as the critical effect and as a basis for the derivation of a recommended limit value for VOCs in indoor air. The committee estimated the maximum tolerable pollution of indoor air by VOCs to be between 0.2 and 3.0 mg/m³ and recommended a limit value of 0.2 mg/m³ for VOCs as a mixture (*106*). The committee emphasized that this value does not take into

account potential health risks attributable to individual VOCs with known carcinogenic, reprotoxic, or sensitizing properties. VOCs possessing such properties should not be used in building materials, and when their use is unavoidable, a separate risk assessment should be performed.

*Harmonized hazard classification criteria for mixtures.* In November 1994, the 22nd Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the Organisation for Economic Co-operation and Development (OECD) created the Programme on Harmonization of Classification and Labelling (HCL). The objective of this program was to develop a harmonized classification system for chemical substances and mixtures. The work on this classification system for mixtures comprised the following eight hazard end points: acute toxicity, skin and eye corrosion/irritation, respiratory or skin sensitization, germ cell mutagenicity, carcinogenicity, reproductive toxicity, specific target organ systemic toxicity, and hazards for the aquatic environment. A detailed review document (DRD), "Hazard Classification for Chemical Mixtures in OECD Countries," was developed and formally approved by the 9th Meeting of the Task Force on HCL in February 2000 (*108*). This DRD is available as document ENV/JM/HCL(99)10/REV2 Part 1 and Part 2. The DRD on mixtures was further discussed, and drafting groups prepared chapters on each of the eight hazard end points and a chapter titled "General Introduction and Considerations" (*108*). In September 2000, the 10th Meeting of the Task Force on HCL reached full consensus on the chapter "General Introduction and Considerations" and on all but two chapters on the various hazard end points. The two outstanding chapters were on skin and eye corrosion/irritation and hazards for the aquatic environment. Following two joint meetings of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology, one held in November 2000 and the other in June 2001, the final version of the "Harmonized Hazard Classification Criteria for Mixtures" was published as part of the document "Harmonized Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures" in June 2001 (*109*).

## Discussion

The present survey deals with a variety of mixture studies ranging from the development of a new computer program (*35*) and a new mathematical model as the foundation for rules predicting the toxicity of mixtures (*40*) to the application of gene expression technologies for detecting joint or interactive effects (*34,69*). Moreover, risk assessment of real-life mixtures such as the simultaneous intake of food additives (*92*), combined exposure to fine particles and gases in ambient air (*46–57*), and DBPs in drinking water (*77,79,80*) were addressed. Attention was also drawn to strategies for estimating public health effects of large airports (*81,82,86*) and the development of harmonized hazard classification criteria for chemical mixtures (*108,109*).

The topics discussed varied greatly, which is not surprising because humans are exposed concurrently and sequentially to hundreds of thousands of chemicals from very different sources such as food, drinking water, beverages, indoor and outdoor air, soil, and consumer products. Thus, mixed exposures are everywhere and are the rule rather than the exception, indicating exposure assessment, hazard identification, risk assessment, and risk characterization should focus on mixtures rather than on single chemicals. However, until recently, about 95% of the sources in toxicology were devoted to the exception, namely, single compounds (*110,111*). Although all of this is true, there is an alternative way to look at exposure to chemical mixtures. Humans (and animals) apparently have learned to cope with simultaneous exposure to huge numbers of chemicals. In fact, exposure to certain mixtures, for example, mixtures of essential nutrients, drinking water, and air, are vital. Moreover, one might wonder whether ideally our food should be exclusively composed of a mixture of pure essential nutrients, and our drinking water of pure $H_2O$, and the air we breathe of pure oxygen or a mixture of pure oxygen and nitrogen. Such pure environments are unrealities and therefore should not be pursued. This implies that the focus in toxicology should not be on mixtures (and chemicals) but on priority mixtures (and priority chemicals), with priority being determined by (potential) risk (= toxicity and exposure), i.e., the smaller the (presumed) margin of safety (the ratio exposure level to health-based limit value might even be >1), the higher the priority. To set such priorities, choices have to be made on the basis of data or educated guesses. Because resources for (mixture) research are limited, risks based on perception only should not be considered and realism should outweigh purism.

Finally, we draw attention to the work of an ad hoc Committee of the Health Council of the Netherlands that has just finalized a report on assessment of health effects of exposure to combinations of substances (*112*). The report presents a framework for health risk assessment of exposure to combinations of chemicals. Two conspicuous elements of this framework are the distinction between mixtures and specified combinations of substances, and the use of the "top *n*" and "pseudo top *n*" approaches. For prioritization of mixtures or combinations of chemicals, the report also includes the Mumtaz and Durkin WOE approach (*113,114*).

This overview highlights some international issues on the toxicology of mixtures. Clearly, strategies to tackle the safety evaluation of combined exposures and complex chemical mixtures, as well as models facilitating the interpretation of findings in the context of risk assessment of mixtures, have become increasingly important.

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