10

Exposure Biomarkers

Larry L. Needham, James Bond, and Steve Tannenbaum

ABSTRACT: This workshop was designed primarily to examine available technologies for screening all or a selected portion of the approximately 72,000 chemicals that are included in the Toxic Substances Control Act (TSCA) for health effects in humans and, to a lesser degree, their effects on the ecological system. In terms of risk assessment, such screening procedures would yield data for use in hazard identification. Obviously, in order to screen this immense number of chemicals, the chemicals must be prioritized. One such focus could be on the 14,000 non polymeric TSCA Inventory chemicals produced in amounts greater than 10,000 pounds per year. Nonetheless, screening all of these 14,000 chemicals for various health endpoints still requires that they be further prioritized. No doubt, quantitative structure activity relationships will be used to set priorities. However, we submit that priority setting could also be based, at least in part, on another aspect of risk assessment - human exposure assessment, for without human exposure, there are ***no*** *adverse health effects, and no need would exist for further risk characterization. Human exposure has been assessed by a variety of means. We believe that the most accurate means of assessing human exposure is the measurement of biomarkers of exposure in human specimens.*

In this presentation, we give examples of how using such biomarkers provided qualitative and quantitative exposure information that proved useful in conducting epidemiological studies. We also present how reference range levels of exposure biomarkers in humans (as acquired by biomonitoring programs) have been extremely beneficial in conducting exposure assessments and how expansion of such programs would directly benefit TSCA. Programs, such as the National Health and Nutrition Examination Survey (NHANES) and the National Human Exposure Assessment Survey (NHEXAS), are available to collect and bank the needed specimens. Analytical methods would then be used in these programs to determine whether, and to what extent, humans were being exposed to particular TSCA-related substances. If so, more extensive "effect screening" methodologies would be used for these substances; if no, or little exposure, was detected, these substances may be given a low priority for "effect screening", and further risk characterization.

Humans are exposed daily to a variety of chemicals that are present in the environment as pollutants or that are in commercial products. For this presentation, we shall assume that these chemicals are included in the Toxic Substances Control Act (TSCA) list of some 72,000 chemicals. Humans are exposed when they come into contact for an interval of time with such chemicals in an environmental medium–soil, water, air, or food or in another medium, such as a commercial product, or in an occupational setting (16, 21, 23, 26). Epidemiologists, risk assessors, and others often classify the degree of exposure or potential exposure, by using the concentration of a given chemical in the media that humans contact, integrated over the time of contact; this is then the basis of an exposure index (18). When humans have contact with these environmental media, the chemical may enter the body via inhalation, ingestion, and/or skin absorption. Once in the body, the chemical may distribute to tissues, and adverse health effects may result.

The amount of chemical absorbed in body tissue is called the internal dose. Common measures used to determine internal dose are the blood and urine levels of chemicals or their metabolizes (23). A portion of this internal dose may reach and interact with a target site over a given period so as to alter physiologic function; this portion is called the biologically effective dose (23). All exposure and dose terms are further defined by Sexton et al. (26). Various methods have been used to assess human exposure to xenobiotics. Our charge is to concentrate on the use of biomarkers in exposure assessment. We do this by presenting case studies which demonstrate the benefits of using biomarkers as opposed to those exposure indices that do not use biomarkers of exposure. We then describe biomonitoring programs and analytical methods that may be beneficial to priority setting in TSCA. In the

following paragraphs, we critique various methods that have been used to assess human exposure.

EXPOSURE INDEX (WITHOUT BIOMARKERS)

Traditionally, exposure has been assessed by estimating the individual's or population's potential for exposure. If the concentration of a given chemical in various media is known, then the total concentration of that chemical in the environmental media that humans contact, integrated over the time of contact, forms the basis of an exposure index. The concentration of the chemical in the environmental media is sometimes based on analytical measurements of environmental samples – water, air, soil, food – collected at the exposure site near or as to close to the time of exposure as possible. Depending on the pathway of exposure, all of these environmental media, and perhaps multiple samples of each, may have to be analyzed at a high cost and yet may not be representative of the concentration of the chemical in the media at the time of human exposure. For example, is the average level of a pollutant in fish caught in a river representative of all such fish in that river? Perhaps the "best" environmental sample for an airborne chemical would be a personal air sample collected at the time of exposure by an organic vapor badge; in one experiment this technique correlated more highly with blood levels of selected volatile organic compounds (VOCs) than did VOC levels in breathing zone air that was collected by charcoal tubes (12). Clearly, such techniques are only available in designed experiments. The estimated time of contact, including frequency and duration, with the environmental medium containing the chemical is generally collected by questionnaire or information obtained during history taking. This combination of questionnaire/history information and environmental measurements are then weighted into an exposure model, which is used as an estimate of exposure for each person. We call this the "environmental approach" for assessing exposure.

This approach may be useful in human exposure assessment as a preliminary screen to help ascertain the potential for human exposure. Various models have been used for both qualitative and quantitative predictions. However, they are based on a plethora of assumptions that may contain several potential problems, such as the inability to adjust for individual factors that relate to how much chemical enters the body and how much is absorbed (individual metabolism differences, individual nutritional status during exposure, individual differences in surface area or body mass, and personal habits such as hand-to-mouth activities). In addition, the frequency and duration of contact with the environment that contains the chemical are difficult to estimate because of uncertainty of recall or bias in administering and answering the questionnaire. This bias may arise whenever non comparable information is obtained from the different study groups, a factor that may be the result of the interviewer eliciting or interpreting the information differently (interviewer bias) or of the participants either intentionally or unintentionally reporting the events in a non comparable manner (recall bias). For example, participants may have problems recalling the frequency of playing on contaminated soil or consuming a certain food. Thus, we believe that such exposure indices are useful but are not the best means to assess human exposure to environmental chemicals.

By definition, the best measure of exposure for assessing dose-response relationships is the biologically effective dose. Ideally, environmental health scientists would like to have sensitive and specific measurements of the biologically effective dose. However, identifying the target site(s) of the chemical is a major impediment to using measures of the biologically effective dose to quantify exposure. Even when the target site is known, an invasive procedure may be required to sample that site (e.g., liver, brain). Some organic toxicants or their metabolizes covalently bond to DNA, thus forming a DNA adduct; most notably, carcinogens and mutagens form such adducts. The measurement of such adducts is called the biologically effective dose, but the levels of these

adducts may reflect only recent exposure because of DNA repair. Likewise, measurements of adducts with hemoglobin and other proteins, such as albumin, have also been considered measurements of the biologically effective dose, and as exemplified by 4-amino biphenyl, the hemoglobin adduct has been shown to be significantly associated with DNA adduct concentration in the human bladder epithelial cells (21). Some of these adducts are specific markers for a toxicant (e.g., benzo(a)pyrene in lymphocytes), whereas others are much less specific (e.g., DNA adducts with alkyl groups). The measurement of adducts in humans is still in the developmental stage, and for most chemicals, much more information is needed before the biologically effective dose can be used as a quantitative measurement of exposure (28). Nonetheless, it can be used as a marker of exposure. Other disadvantages to be considered in these measurements are that sample throughput may be too low for moderate-size epidemiological studies, and many adducts may arise from a single chemical.

The next most useful exposure measures are those of internal dose. The direct measurement of a chemical or one of its metabolizes in blood or urine has significantly improved human exposure assessment and thus has improved assessing the risk to humans of many important chemicals. For example, it is fair to say that without blood lead measurements, most of the central nervous system effects of low-level lead exposure could not have been detected.

To interpret blood or urine chemical levels accurately, analysts must know the pharmacokinetics of the chemical and also must have a knowledge of the background levels found in the general population. For example, some chemicals, such as VOCs, are rapidly eliminated, whereas others, such as the chlorinated hydrocarbon pesticides, may have a half-life in humans of greater than 5 years. Thus, such information is critical for interpreting whether the measured concentration of a chemical reflects recent exposures, long-term (chronic) exposures, or both. Of course, to the extent possible, it is still of great importance for the epidemiologist to collect, non-biased information from study participants regarding their potential exposure.

Additional biomarkers that have been monitored in humans include biomarkers of susceptibility and effect. Biomarkers of response, such as cytogenetic markers, stress proteins, and enzyme induction, are sometimes classified as exposure biomarkers and sometimes as effect biomarkers. We will not consider them in this presentation because of space limitations but more importantly because these biomarkers are very nonspecific; i.e., abnormalities of these biomarkers would not specify to what TSCA chemical one may have been overtly exposed, if any.

EXAMPLES OF USE OF BIOMARKERS IN EXPOSURE ASSESSMENT

We will now demonstrate how biomarkers of exposure have been used in exposure assessment in epidemiological studies and how this approach is preferred over the "environmental approach" for assessing human exposure. In so doing, we do not wish to imply that the environmental approach is meaningless, but that the biologic approach is preferred as a marker of human exposure. Certainly, in risk management when the objective is to reduce the potential exposure, the "environmental approach" is useful for identifying where the pollution is taking place.

Dioxin: Operation Ranch Hand Study

From 1962 through 1970 during the Vietnam Conflict, the main mission of the U.S. Air Force's Operation Ranch hand was to spray defoliants, such as Agent Orange, over densely vegetated areas of South Vietnam. Agent Orange consisted of an equal mixture of 2,4-D and 2,4,5-T in diesel oil; the 2,4,5-T was contaminated with 2,3,7,8-TCDD (dioxin) in the parts-per-million range. Dioxin is lipid soluble and thus tends to be stored in the lipid-rich depots of the human body. Dioxin has a long half-life-more than 7 years in humans (20, 25). In 1982, the Air Force began a prospective cohort study, specifically looking at health, reproductive, and mortality outcomes that might be associated with exposure to Agent Or-

ange and other herbicides containing dioxin. These health studies will examine the veterans of Operation Ranch Hand every 5 years through the year 2002. One of the first tasks was to develop an exposure index in order to classify each veteran's exposure; this index would then be used as the basis for exposure and for correlating with any health effects.

This exposure scenario was similar to that of exposure in an occupational setting in that the primary exposure was thought to be direct exposure to the herbicide itself, rather than indirect exposure through an environmental pathway. The exposure index consisted of the average concentration of dioxin in the Agent Orange during one's tour of duty multiplied by the number of gallons of Agent Orange sprayed during one's tour divided by the number of men in one's specialty during that period. The total number of eligible men in the study was limited to the 1200 to 1300 survivors of the Operation. The U.S. Air Force and various review boards believed that this index not only could serve as a reliable basis for assessing exposure to dioxin but that any noted adverse health effects could be related to this index.

In 1987, the U.S. Air Force contracted with our laboratory to analyze 150 serum samples from Operation Ranch Hand veterans in order to compare the Air Force's exposure index with the measured internal dose of the veterans. There was essentially no correlation between the exposure index and the serum dioxin level (14). Because of this finding, the Air Force further contracted with CDC to analyze the serum of all surviving members of Operation Ranch Hand, and this serum-dioxin level became the exposure index used to correlate with any adverse health effects (33). Had the Air Force used its original exposure index for the Operation Ranch Hand study, a great deal of misclassification would have resulted, and any health effect conclusions of the study would have been invalid.

Thus, the use of the serum dioxin measurement, the biomarker, was preferred over the exposure index that was derived without the biomarker.

Dioxin: U.S. Army Ground Troops in Vietnam

The chemical of concern was again the dioxin in Agent Orange. The potential environmental pathways were skin contact with and inhalation of the spray containing the herbicide, skin contact with sprayed vegetation and soil, and ingestion of water and food that had been sprayed. The amount of dioxin in the Agent Orange from 1966-1969 was known. The duration of contact was gathered from questionnaires given to the veterans and from U.S. military records containing the locations of military units, the locations where herbicide was sprayed, and the dates when the herbicide was sprayed.

Six exposure indices were generated from this information; four of the indices were based on a soldier's potential for exposure from direct spray or on his being located in an area that had been sprayed within the previous 6 days; the other two exposure indices used self-reported data and included an index that was based on the veteran's perception of how much herbicide he has been exposed to. To test the validity of these exposure indices, CDC measured serum dioxin levels in 646 enlisted ground troop veterans who had served in III Corps a heavily sprayed area, for an average of 300 days during 1966 to 1969. For comparison, serum-dioxin levels in 97 non-Vietnam U.S. Army veterans who served during the same time were also measured (30).

The results showed no meaningful association between dioxin levels and any of the exposure indices. The mean, median, and frequency distributions for both the Vietnam and non-Vietnam veterans were remarkably similar, indicating that there was little, if any, increased exposure to dioxin in this population. The study had a 95°/0 statistical power to detect a difference of only 0.6 ppt in the medians, but this difference was not found. This finding exemplifies the value of measurements of internal dose in exposure assessment. It also points out the need to develop specific and sensitive methods, for if the detection limit for dioxin had been 20 ppt (lipid adjusted), then most all the results would have been non detectable. Furthermore, because elevated expo-

sures could not be documented, plans for a prospective cohort health study were dropped.

Dioxin: Occupational Setting

CDC National Institute of Occupational Safety and Health (NIOSH) conducted a retrospective study to evaluate health outcomes, including mortality from cancer, among more than 5000 workers who may have been occupationally exposed to dioxin, as a result, for example, of the production of 2,4,5 -trichlorophenol (9). Many of these workers were deceased. Because many were deceased and because of the large number of potentially exposed men, NIOSH epidemiologists had to develop an exposure index for use in correlating the health outcomes (the effect). Serum dioxin measurements were performed on 253 workers; the results were compared to various exposure indices. From this analysis, epidemiologists determined that the best exposure index was years of work in a job with potential exposure. Since this exposure index had been validated to and calibrated with serum dioxin levels, it could be used as the exposure index in this study and exposure and effects could be compared directly with those found in other studies. This process again demonstrates the need for measuring the internal dose in exposure assessment or health effect studies.

Lead

Toxicity associated with high levels of lead in humans has long been recognized. However, biochemical and epidemiological studies have noted hematological and neurological damage among children with relatively low levels of lead in their blood and teeth. The second National Health and Nutrition Examination Survey (NHANES II), conducted by the National Center for Health Statistics (NCHS), provided blood lead measurements, which were the basis for estimating the degree of exposure of the general U.S. population to lead (1). As a result of federal regulations requiring the removal of lead from gasoline, the amount of lead in gasoline decreased about 55°/0 from early 1976 to early 1980. The population-based NHANES II Study showed that the predicted mean blood level in the U.S. population had decreased 37% during that same period, from 14.6 µg/dL to 9.2 µg/dL. Environmental modeling did not accurately predict the magnitude of the impact of decreasing the amount of lead in gasoline because the contribution of lead from gasoline to humans via the soil was not well characterized. These data were a major factor in the Environmental Protection Agency's (EPA's) decision to implement a more rapid removal of lead from gasoline. This implementation and the banning of the lead-soldering of cans produced in the U.S. have been major factors in the NHANES III predicted mean blood level decreasing to 2.8 µg/dL in the U.S. population in 1990 (24).

Thus, exposure assessment by measuring blood lead levels has been a public health success story. It helped identify lead in gasoline as a major preventable source and showed that removing lead from gasoline was an effective prevention strategy. However, the latest data indicate that 8.9%, or approximately 1.7 million children, aged 1-5 years, have blood lead levels equal to or greater than 10 µg/dL, which is the level of concern under the 1991 CDC guidelines. The population at risk for excessive lead exposure comprises primarily black, inner-city children and has been targeted for more extensive lead poisoning prevention efforts (6). This example again shows the need for biomarkers of exposure for relating exposure to health effects.

Volatile Organic Compounds (VOCs)

Many volatile organic compounds (VOCs) are ubiquitous in the environment. They have been shown to exist in higher concentrations in indoor air than in outdoor air (32). Reported health effects from exposure to VOCs have included eye irritation, sick-building syndrome, neurological effects, and cancer. CDC developed an isotope-dilution purge and trap gas chromatography/mass spectrometry method to quantity 32 VOCs (see table 10-1) in 10 mL of blood with detection limits in the parts-per-trillion range (3). This method is a full-scan method at 3000 resolving power, so that in addition to acquiring quantitative data on these 32 VOCs, many additional VOCs can be

Table 10-1 Biological Monitoring at CDC's National Center for Environmental Health

Metals (typical urine or blood sample -3 mL; typical limit of detection - low parts per billion (ppb)

Lead	Beryllium	Arsenic
Mercury	Chromium	Thallium
Cadmium	Nickel	Vanadium

Polychlorinated dibenzo-dioxins, polychlorinated dibenzo-furans, coplanar polychlorinated biphenyls (PCBs) **(measured in serum from one 25 mL blood sample if exposure is near background levels - smaller samples are adequate** for higher exposures; typical limit of detection - low parts-per-trillion (ppt) on a lipid-weight basis, low parts-per-quadrillion on a whole-weight basis)

2,3,7,8-Tetrachlorodibenzo-p-dioxin	(TCDD)	1,2,3,4,7,8-Hexachlorodibenzofuran	(H_xCDF)
1,2,3,7,8-Pentachlorodibenzo-p-dioxin	(P_nCDD)	1,2,3,6,7,8-Hexachlorodibenzofuran	(H_xCDF)
1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin	(H_xCDD)	1,2,3,7,8,9-Hexachlorodibenzofuran	(H_xCDF)
1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin	(H_xCDD)	2,3,4,6,7,8-Hexachlorodibenzofuran	(H_xCDF)
1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin	(H_xCDD)	1,2,3,4,6,7,8-Heptachlorodibenzofuran	(H_pCDF)
1,2,3,4,6,7,8-Heptachlorodibenzo-pdioxin	(H_pCDD)	1,2,3,4,7,8,9-Heptachlorodibenzofuran	(H_pCDF)
1,2,3,4,6,7,9-Heptachlorodibenzo-p-dioxin	(H_pCDD)	1,2,3,4,6,7,8,9-Octachlorodibenzofuran	(OCDF)
1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin	(OCDD)	3,3',4,4'-Tetrachlorobiphenyl	(TCB)
2,3,7,8-Tetrachlorodibenzofuran	(TCDF)	3,4,4',5-Tetrachlorobiphenyl	(TCB)
1,2,3,7,8-Pentachlorodibenzofuran	(P_nCDF)	3,3',4,4',5-Pentachlorobiphenyl	(P_nCB)
2,3,4,7,8-Pentachlorodibenzofuran	(P_nCDF)	3,3',4,4',5,5'-Hexachlorobiphenyl	(H_xCB)

Volatile organic compounds (VOCs) (measured in one 10 mL blood sample; typical limit of detection - low ppt)

1,1,1 -Trichloroethane	Acetone	Ethylbenzene
1,1,2,2-Tetrachloroethane	Benzene	Hexachloroethane
1,1,2-Trichloroethane	Bromodichloromethane	m-/p-Xylene
1,1-Dichloroethane	Bromoform	Methylene chloride
1,1-Dichloroethene	Carbon Tetrachloride	o-Xylene
1,2-Dichlorobenzene	Chlorobenzene	Styrene
1,2-Dichloroethane	Chloroform	Tetrachloroethene
1,2,-Dichloropropane	cis-1,2-Dichloroethene	Toluene
1,3-Dichlorobenzene	Dibromochloromethane	trans-1,2-dichloroethene
1,4-Dichlorobenzene	Dibromomethane	Trichloroethene
2-Butanone		

Chlorinated pesticides and non-coplanar polychlorinated biphenyls (measured in serum from one 5 mL blood sample; typical limits of detection - low ppb)

Aldrin	Biphenyls, Polychlorinated (congeners)	Endrin
Chlordane, alpha	DDD	Heptachlor
Chlordane, gamma	Trans-Nonachlor	Heptachlor epoxide
beta-Hexachlorocyclohexane	DDE	Hexachlorobenzene
gamma-hexachlorocyclohexane	DDT	Mirex
Biphenyls, Polychlorinated (total)	Dieldrin	Oxychlorodane

Non-persistent pesticides (measured in one 10 mL urine sample typical limits of detection - low ppb)

Urine metabolites	Parent Pesticides
2-Isopropoxyphenol (IPP)	Propoxur
2,5-Dichlorophenol (25DCP)	1,4-Dichlorobenzene
2,4-Dichlorophenol (24DCP)	1,3-Dichlorobenzene, Dichlofention, Prothiofos, Phosdiphen
Carbofuranphenol	Carbonfuran, Benfuracarb, Carbosulfan, Furanthiocarb
2,4,6-Trichlorophenol (246TCP)	1,3,5-Trichlorobenzene, Hexachlorobenzene, Lindane
3,5,6-Trichloro-2-pyridinol (TCPY)	Chloropyrifos, Chlorpyrifos-methyl
4-Nitrophenol (NP)	Parathion, Methyl parathion, Nitrobenzene, EPN
2,4,5-Trichlorophenol (245TCP)	1,2,4-Trichlorobenzene, Fenchlorphos, Trichloronate, Lindane
1-Naphthol (1 NAP)	Naphthalene, Carbaryl
2-Naphthol (2NAP)	Naphthalene
2,4-Dichlorophenoxyacetic acid (24D)	2,4-D
Pentachlorophenol (PCP)	Pentachlorophenol

qualitatively identified and in many cases, quantified (5).

CDC, with financial support from the Agency for Toxic Substances and Disease Registry (ATSDR), selected a 1,000 person subset of the NHANES III population to determine reference ranges for these 32 VOCs. The 1,000 people were chosen from both sexes, all regions of the contiguous U. S., urban and rural residents, and were adults between 20 and 59 years of age (19). The data showed that 11 of these VOCs were measured in more than 75°/0 of the people with the non chlorinated aromatics being the most prevalent. These VOCs included styrene, toluene, ethyl benzene, o-xylene, m,p-xylene, and benzene, which is a known human carcinogen. The primary sources of these compounds are tobacco smoke and exhaust from internal combustion engines. The non endogenous compound found at the highest concentration and highest frequency was 1,4-dichlorobenzene (4). The blood exposure data for this moth repellent and room deodorizer correlated highly with urinary levels of its primary metabolize, 2,5-dichlorophenol (1 1). This high correlation indicated that either blood 1,4-dichlorobenzene or urinary 2,5-dichlorophenol levels could be used as a biomarker of exposure to 1,4-dichlorobenzene.

Five of the VOCs were found in 10%-75% of the selected population, whereas the remainder of the VOCs were in less than 10% (4). Thus, this latter group would be of low priority for inclusion in human effect studies. These analytical methods and reference range studies have been applied to a wide variety of case studies and population studies. These include exposure assessment studies of toxic waste sites, oil-well fires (7), sick building syndrome (4), multiple chemical sensitivity, and oxygenated fuels involving methyl tertiary-butyl ether (MTBE) (15). In each of these examples, the blood concentrations of VOCs were compared with the reference-range population data. However, pharmacokinetic data are needed to properly interpret blood levels of VOCs. Scientists from CDC and EPA have collaborated in determining the half-lives of many VOCs in humans subjected to low level mixtures of VOCs in well-controlled chamber studies (2). The blood half-lives were less than one-half hour, but the elimination time courses were multiexponential, thereby suggesting multiple storage sites within the body. The blood uptake portion of the 4-hour exposure curve exhibited a rapid uptake that reached a plateau after about 50 minutes; the uptake rate was not concentration dependent, but the blood concentration was directly dependent on the air concentration. When exposure ceased after 4 hours, the decay was rapid, but the decay rate also reached a plateau after about 1 hour; however, the VOC levels remained elevated even 24 hours after exposure as compared with the pre-exposure blood levels. Thus, like those compounds with long biologic half-lives, such as dioxin, VOCs also can be the focus of exposure assessment studies, if the blood samples are collected within 1 day following exposure.

COLLECTING AND BANKING OF HUMAN SPECIMENS

We have presented examples of the benefits of biomarkers of exposure; now we focus on the mechanisms of collecting and banking human specimens for such biomonitoring. The first U.S. program of biological monitoring tissue specimens for environmental pollutants and also for human tissue specimen banking was the National Human Monitoring Program (N-IMP), which began in 1967 and was conducted by the U.S. Public Health Service. When the U.S. Environmental Protection Agency (EPA) was created in 1970, the NHMP was transferred to it. One of the major activities of the NHMP was the National Human Adipose Tissue Survey (NHATS), which was designed to be a continuously operating survey that would collect, store, and analyze autopsy and surgical specimens of human adipose tissue from the major U.S. metropolitan areas. However, during the 1980s, budget cuts restricted the NHMP to a reduced and modified NHATS, which continued until 1990. In 1991, the National Research Council published its findings that programs that provide more useful data based on

probability samples for the entire U.S. population should be designed and properly funded (1 7).

One program that is based on a national probability sample is the National Health and Nutrition Examination Survey (NHANES), which is conducted by the Centers for Disease Control and Prevention's National Center for Health Statistics. Data from NHANES I, II, and 111 have provided important information on the prevalence of various health conditions and distributions of physical and biochemical characteristics of the U.S. population. As previously mentioned, data on blood lead levels in NHANES II and III provided longitudinal trend data on human levels and the effect of legislation on that trend. The data also pinpointed a sub population still at risk for excessive lead exposure. Serum levels of cotinine, the major metabolize of nicotine, are being measured in NHANES III in order to ascertain exposure levels as a result of both active and passive smoking (29). As mentioned previously, CDC measured blood VOCs and selected urinary pesticide residues in a subset of the NHANES population in order to assess human exposure to these compounds. In addition, blood, urine, and DNA have been banked from NHANS III.

Phase I of the National Human Exposure Assessment Survey (NHEXAS), which is conducted under cooperative agreements with the EPA, began in 1995. These Phase I studies are population based surveys for exposure assessment to selected environmental pollutants in the state of Arizona and in EPA's Region V (29).

Designing and implementing national probability sampling surveys for human exposure assessment must consider many issues (8). However, certainly NHANES, and now NHEXAS, have addressed these issues. Therefore, the mechanism is in place to collect and bank specimens needed to assess biomarkers of exposure in human specimens for many of the chemicals included in TSCA.

PRIORITIZING CHEMICALS

We have presented examples of the benefits of biomarkers of exposure and the ability of programs, like NHANES, to collect and bank the needed biologic specimens for assessing human exposure to many of the chemicals included in TSCA. This does not argue that the entire number of probability based samples have to be analyzed but that mechanisms are in place to collect such samples. Assuming the needed biologic samples are available, the list of TSCA chemicals must be prioritized for the effective application of biomarkers for human exposure assessment. The following factors would be included in such prioritization:

- potential for human exposure
  - degree of exposure
    - pounds produced per year
    - physical/chemical characteristics of chemical
    - how the chemical is made, used, fate
  - number of people potentially exposed
  - susceptible population
- hazard identification/severity of effect information
- dose/response information in both animals and humans
- possibility of measuring biomarkers

Such prioritization of this chemical list would therefore involve development of a model that would include the following factors: the potential for human exposure (degree and number), severity of adverse effects in a dose response manner, and the possibility of the biomarkers existing and ability of the laboratory to develop the needed analytical methods. For those chemicals that lack the needed information, quantitative structure activity relationship data, if available, would also be used. Exposure databases (1 O) would be used in this process.

ANALYTICAL METHODS

As mentioned previously, one of the criteria for prioritizing the list of chemicals for the development of biomarkers is the possibility of measuring biomarkers of exposure; i.e., does a biomarker exist and can the laboratory develop the needed analytical methods to measure the biomarker? Unless the biomarker exists, there is

no need for the analytical method. Assuming the biomarker exists, the analytical methods should have the following characteristics:

- Multianalyte (several biomarkers)
- Compatible with sample matrix
- Demonstrated acceptable sensitivity
- Demonstrated acceptable specificity
- Demonstrated acceptable precision
- Demonstrated acceptable accuracy
- Cost effective
- Rapidity

These characteristics, except for cost effective and rapidity, can be defined in objective terms. Certainly, the methods used in our examples meet the needed objective criteria. For measuring organic biomarkers of exposure, the analytical methods that are atop the method hierarchy include high resolution mass spectrometry and tandem mass spectrometry using the isotope dilution technique for quantification. Whether a particular analyses is cost effective is more subjective. For example, the cost for the measurement of 32 VOCs in 10 mL of Blood is about $500 per sample or less than $20 per analyte. Commercial prices for measuring the 17 polychlorinated dibenzo-p-dioxins and furans plus 4 co-planar polychlorinated biphenyls that are in human serum are about $1000 per sample or less than $50 per analyte. One can decide if this is too costly for the intended purpose.

Historically, mass spectrometric methods have suffered in the area of rapidity or high throughput, but this is not always the case. For example, for the measurement of cotinine in NHANES III, serum extracts are analyzed at the rate of 1 every 2 minutes by using high performance liquid chromatography/atmospheric pressure ionization tandem mass spectrometry. This technique also requires less sample preparation than traditional methods although sample preparation is the rate limiting step because of the speed of mass spectrometric analysis.

Other methods which may appear to be more amenable to screening methodologies; i.e., low cost and rapid, have been developed for many chemicals, primarily pesticides, in the environmental area (15, 31). To expand this list to many of the TSCA chemicals in biological specimens would require much work in both developing the antiserum and the methods. Many of the current immunoassay have high levels of false positives (because of cross-reactivity or matrix effects) and false negatives (because of matrix effects unless sufficient sample preparation procedures are followed). Therefore, frequently to meet the objective requirements of the desired analytical methods, one must employ methods of higher specificity for many of the samples. One new technique that employs many of the advantages of immunoassay with the specificity and multianalyte capability of the mass spectrometer is a mass spectrometric immunoassay (15). Such combinations of techniques will be used increasingly for biomonitoring.

We believe that the bottom line is that following some prioritization of the chemicals, if the biomarker of exposure exists in a readily accessible biologic specimen, such as blood or urine, this biomarker can be measured effectively to assess human exposure and thus be used to help prioritize TSCA chemicals for health effect screening. The converse that biomarkers of effect can help prioritize chemicals for exposure studies is also true.

SUMMARY

We have attempted to show that a biomonitoring program would be beneficial in assessing human exposure to many of the chemicals on the TSCA list. Such a program might be also a way to 1) establish reference ranges in the general population; 2) identify sub populations potentially at risk; 3) establish trends in exposure and, hence, judge the effectiveness of pollution prevention practices and regulations; 4) provide dose assessment over total exposure; 5) and provide a data base for comparison with other data sets such as ecological data sets. The needed sample collection programs and analytical procedures are now available for conducting such a program. These procedures incorporate the benefits of having the required sensitivity, specificity, and

multi-chemical measurements and are cost effective. National population-based programs such as the National Health and Nutrition Examination Survey (NHANES) or the National Human Exposure Assessment Survey (NHEXAS) could be used to collect the specimens. Each of these would offer certain advantages. The TSCA list would have to prioritized by using an algorithm consisting of the potential for human exposure, severity of adverse human effects and the possibility of measuring the required biomarker. Once this model is formed, it could be validated by the biomonitoring program.

We have also included a list of the chemicals (table 10-1), for which CDC has national human internal dose data, the biologic specimen needed and amount, and the lower detection limits; these data are from various sources and are of varying quality for predicting national mean and ranges of human levels. Nonetheless, they do show whether exposure is common for particular chemicals. In addition, many of these chemicals, such as the pesticides, are not on the TSCA list.

We believe that there is a hierarchy of means to assess human exposure. This hierarchy includes self reports, professionally-developed exposure questionnaires, measurements of external dose, and modeling of all or portions of these data. All of this information may be useful, but we believe that the "gold standard" is the measurement of a biomarker of exposure in human specimens. Thus, if exposure data and classification from any of the other techniques are to be used, they should be both validated and calibrated to human biomonitoring data. However, programs such as NHANES or NHEXAS and many of the analytical methods are available to gather exposure information on many TSCA chemicals. This exposure information would then be used to determine which chemicals should be examined for health effects, for without a receptor population, there would be little need to study associated health effects.

REFERENCES

1. Annest, J. L., et al., "Chronological Trend in Blood Lead Levels Between 1976 and 1980," *New England Journal of Medicine 308:373-1377, 1983.*
2. Ashley, D. L., and Prah, J.D. "Time Dependence of Blood Concentrations During and After Exposure to a Mixture of Volatile Organic Compounds," personal communication,
3. Ashley, D.L., et al., "Determining Volatile Organic Compounds in Human Blood from a Large Sample Population by Using Purge and Trap Gas Chromatography/Mass Spectrometry," *Analytical Chemistry* 64:1021-1029, *1992.*
4. Ashley, D. L., et al., "Blood Levels of Volatile Organic Compounds in a Nonoccupationally Exposed U.S. Population and In Groups of Suspected Exposure," *Clinical Chemistry* 40:1401-1404, *1994.*
5. Benin, M. A., et al., "Measurement of Methyl Tert-Butyl Ether and Tert-Butyl Alcohol in Human Blood by Purge-and-Trap Gas Chromatography-Mass Spectrometry Using an Isotope-Dilution Method," *Journal of Analytical Toxicology* 19:187-191, *1995.*
6. Brody, D.J., et al., "Blood Lead Levels in the U.S. Population," *Journal of the American Medical Association* 272:277-283, 1994.
7. Etzel, R. A., and Ashley, D. L., "Volatile Organic Compounds in the Blood of Persons in Kuwait During the Oil Fires," *Int. Archives in Environmental Health* 47:1-5, 1994.
8. Ezzoti-Rice, T. M., and Murphy, R. S., "Issues Associated With the Design of a National Probability Sample for Human Exposure Assessment," *Environmental Health Perspectives* 103(3):55-59.
9. Fingerhut, M.A., et al., "Cancer Mortality in Workers Exposed to 2,3,7,8 -Tetrachlorodibenzo-p-dioxin," New *England Journal of Medicine* 324:212-218, 1991.
10. Graham, J., et al., "Role of Exposure Databases in Risk Assessment," *Archives of Environ. Health* 47:408-420, 1992.

11. Hill, Jr., R. H., et al., "p-Dichlorobenzene Exposure Among 1,000 Adults in the United States," *Archives of Environmental Health 1995.*
12. Mannino, D. M., et al., "Human Exposure to Volatile Organic Compounds: A Comparison of Organic Vapor Monitoring Badge Levels with Blood Levels," *International Archives of Occupational Environmental Health 67:59-64, 1995.*
13. Meulenbert, E.P., Mulder, E. H., and Stoks, P. G., "Immunoassay for Pesticides," *Environment Science and Tech.* 29:553-561, 1995.
14. Michalek, J. E., "The Value of Epidemiological Studies, " *Applied Industrial Hygiene* 12:68-72, 1989.
15. Moolenarr, R. L., et al., "Methyl Tertiary Butyl Ether in Human Blood After Exposure to Oxygenated Fuel in Fairbanks, Alaska," *Archives in Environmental Health* 49:402-409, *1994.*
16. National Research Council, Committee on Advances in Assessing Human Exposure to Airborne Pollutants, "Principles of Exposure Assessment," *Human Exposure Assessment for Airborne Pollutants: Advances and Opportunities* (Washington, DC: National Academy Press, 1991).
17. National Research Council, *Monitoring Human Tissues for Toxic Substances* (Washington, DC: National Academy Press, 1991).
18. Needham, L. L., "Examples of Measuring Internal Dose for Assessing Exposure in Epidemiological Studies," *Environmental Epidemiology: Effects of Environmental Chemicals on Human Health, Advances in Chemistry Series,* W.M. Draper (cd.) (Washington, DC: American Chemical Society,)
19. Needham, L. L., et al., "The Priority Toxicant Reference Range Study: Interim Report, *Environ Health Perspectives* 103(3):89-94, *1995.*
20. Needham L. L., et al., "Half-Life of 2,3,7,8-TCDD in Serum of Seveso Adults: Interim Report," *Organohalogen Compounds 21:81-86, 1994.*
21. Needham, L. L., et al., "Case Studies of Relationship Between External Dose and Internal Dose," *Journal of Exposure. Analysis in Environmental Epidemiology.* l(Supplement):209-221, 1992.
22. Nelson, R. W., et al., "Mass Spectrometric Immunoassay," *Analytical Chemistry* 67: 1153-1158, 1995.
23. Lioy, P.J., "Assessing Total Human Exposure to Contaminants," *Environmental Science and Technology* 24:938-945, 1990.
24. Pirkle, J. L., et al., "The Decline in Blood Lead Levels in the United States," *Journal of the American Medical Association 272:284-291, 1994.*
25. Pirkle, J. L., et al., "Estimates of the Half-Life of 2,3,7,8-TCDD in Ranch Hand Veterans," *Journal of Toxicology and Environmental Health* 27:165-171, *1989.*
26. Sexton, K., Callahan, M.A., and Bryan, E. F., "Estimating Exposure and Dose to Characterize Health Risks: The Role of Human Tissue Monitoring in Exposure Assessment," *Environmental Health Perspectives.* 103(3): 13-*29, 1995.*
27. Skipper, P.L, and Tannenbaum, S. R., "Molecular Dosimetry of Aromatic Amines in Human Populations," *Environmental Health Perspectives* 102:17-21, *1994.*
28. Skipper, P.L, and Tannenbaum, S. R., "Protein Adducts in the Molecular Dosimetry of Chemical Carcinogens," *Carcinogenesis* 11:507-518, 1990.
29. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, "Preliminary Data: Exposure of Persons Aged Greater Than or Equal for 4 Years - United States, 1988 -1991," *Mortality and Morbidity Weekly Report* 42:37-39, 1993.
30. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, "Veterans Health Studies: Serum 2,3,7,8-Tetrahlorodibenzo-p-dioxin Levels in U.S. Army Vietnam-Era Veterans," *Journal of the American Medical Association* 260:1249-1254, *1988.*

31. Van Emon, J.M., and Lopex-Avilla, V., "Immunochemical Methods for Environmental Analysis," *Analytical Chemistry* 64:79-88, 1992.
32. Wallace, L.A., et al., "The TEAM Study: Personal Exposures to Toxic Substances in Air, Drinking Water, and Breath of 400 Residents of New Jersey, North Carolina, and North Dakota," *Environmental Research* 43:290-307, 1987.
33. Wolfe, W. E., et al., "Health Status of Air Force Veterans Occupationally Exposed to Herbicides in Vietnam, Physical Health," *Journal of the American Medical Association* 264:1824-1831, 1990.