Doi: 10.1111/j.1742-7843.2007.00185.x
 Basic & Clinical Pharmacology & Toxicology, **102**, 100–108

MiniReview

Assessing Developmental Toxicant Exposures via Biomonitoring

Larry L. Needham, Antonia M. Calafat and Dana B. Barr

Organic Analytical Toxicology Branch, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA, USA

(Received July 27, 2007; Accepted October 12, 2007)

Abstract: Most of the developmental effects that populations experience are believed to be linked with their exposure scenario and/or their susceptibility to these exposures. In environmental public health, most studies have focused on exposures to environmental chemicals but certainly other environmental factors and susceptibility factors must be considered. Our laboratory assesses exposure to environmental chemicals by measuring the chemical, its metabolite(s) or chemical adduct(s) in a biological matrix taken from members of the populations of interest (via biomonitoring). To help interpret data from the many uses of biomonitoring and for other purposes in public health, we have determined, and made public, data on the concentrations of environmental chemicals in the general population of the USA. Exposures at critical time periods of development to many of these chemicals have been linked with adverse developmental effects. In this paper, we examine this linkage using several chemicals as examples and providing biomonitoring information for these chemicals in the US population as a whole but also at various life stages.

For decades, the exposure–effect continuum [1,2] has been the template used for tracking selected environmental chemicals from their origin through the environment into human beings where they may contribute to or cause an adverse effect to the exposed population. The adverse effect, if any, is a product of the exposure, including the dose and the pharmacokinetics of the chemical in the body, and the susceptibility (including pharmacodynamics) of the population to that exposure. Factors that influence the pharmacokinetics and/or pharmacodynamics include diet, nutritional status, housing and community conditions, individual concerns, medications, other environmental chemical exposures, general health status including exposure to infectious agents, genetics and demographic characteristics, such as age, race and sex. For adverse developmental effects, these added factors include not only those associated with an affected individual, but also those of the infant's father and mother, especially during pregnancy (fig. 1). Therefore, each of these factors, in unison and cumulated, affects the pharmacokinetics and pharmacodynamics of an environmental chemical within an individual's body and should be considered in studies designed to link environmental chemical exposures with adverse health outcomes [3]. The potential influence of many of these factors can be assessed by such techniques as questionnaires, medical records, diaries, videos and global information systems. Exposures to environmental chemicals – including chemicals found in pesticide formulations, personal care products, household products, other consumer products and products of industrial processes – are often assessed by the aforementioned techniques but also by more quantitative measures, such as environmental monitoring, personal monitoring and biological monitoring (biomonitoring); the latter technique has the advantage of assessing how much of the chemical was actually absorbed into the body following an exposure. In environmental public health studies, exposure information on the general population and on selected study populations is often missing or incomplete. The laboratories in the Division of Laboratory Sciences in the National Center for Environmental Health of the Centers for Disease Control and Prevention (CDC) have a long history of providing biomonitoring data for epidemiological investigations, general population exposure surveys and for other uses of biomonitoring data [4]. Many of these studies involve chemicals that have been linked to adverse developmental outcomes (*vide infra*).

Author for correspondence: Larry L. Needham, Centers for Disease Control and Prevention, Mailstop F17, 4770 Buford Highway, Atlanta, GA 30341-3724, USA (fax +1 770 488 4546, e-mail lln1@cdc.gov).

Timing of exposure

Prenatal and postnatal development is a continuous process (fig. 2). The developmental stage occurring when the dose of the toxicologically active environmental chemical interacts at the target organ (the biologically effective dose) of the individual determines at least, in part, the type and severity of any adverse effect. For early neurodevelopmental effects, direct or indirect insults on the brain are involved. Although the developing brain has been described as being resilient (being plastic/elastic) to low dose environmental chemical

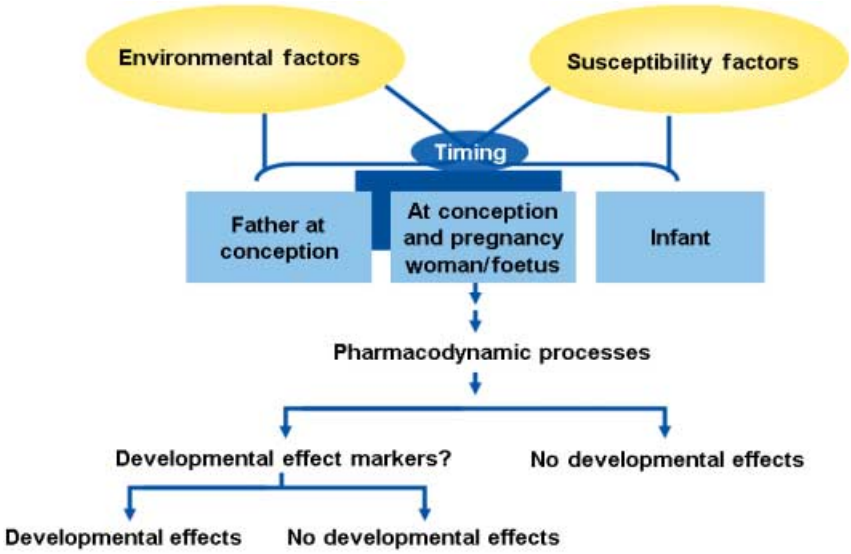

Fig. 1. Interactions of factors potentially leading to adverse health outcomes.

exposures, if these exposures are chronic, then adverse effects that are exhibited early in life or sometimes later in life can occur, especially in those more susceptible because the elasticity of the target organ has been impaired [5–7]. Larger, acute doses may also produce adverse effects but because of the bolus insult these effects may occur independently of the other stress factors and genetics. This may explain in part why exposure to large doses of structurally

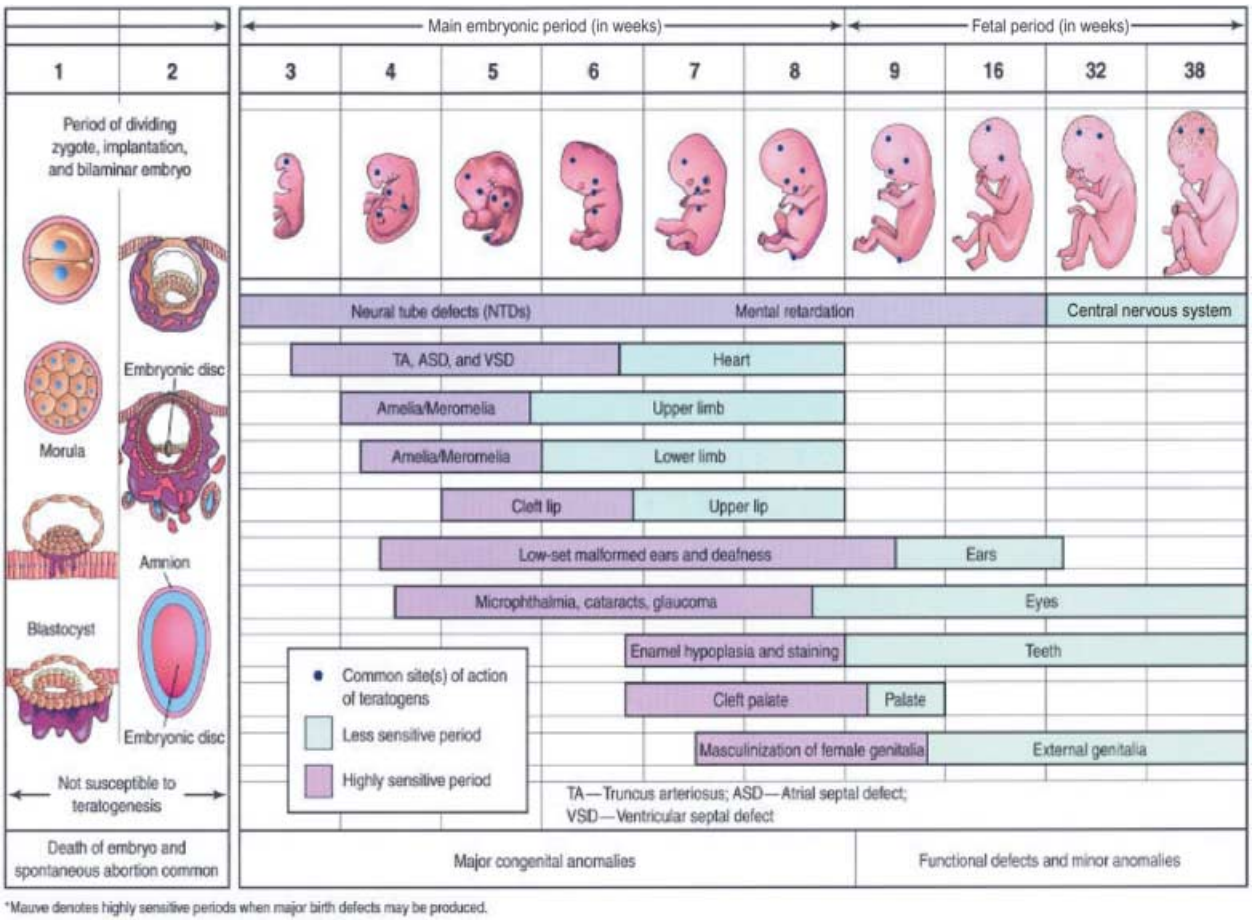

Fig. 2. Critical periods in human development* (from Moore KL, Persaud TVN. The Developing Human: Clinically Oriented Embryology (6th ed). Philadelphia: Saunders, 1998. Used with permission).

different chemicals, received both prenatally and postnally, have been linked to neurological adverse health outcomes of the central nervous system that continues its development for many months after birth [8,9]. The percentages of such effects that are due to stresses at conception prenatally versus postnatally are not known for most chemicals (fig. 1).

A difficulty in studying environmental exposures related to developmental effects is knowing the exposure time period of the greatest concern for a particular adverse outcome. Certainly, developmental time period information like that shown in fig. 2 is of assistance. Although developmental effects may occur from postnatal exposures, most of these effects have been linked most strongly to prenatal exposures (fig. 2). For most environmental chemicals, biomonitoring data on their concentrations in the foetal environment or even during early infancy are scarce. As part of our involvement in the Exposure Workgroup for the National Children's Study [10], we devised a series of tables indicating the preferred biological samples to analyse for assessing exposures to various environmental chemical classes during different human life stages, including the critical foetal developmental period [11]. For example, for the persistent organic chemicals, such as polychlorinated biphenyls and selected organochlorine insecticides, one can estimate foetal exposures by analysing maternal blood and adipose tissue taken during pregnancy or cord blood, assuming that these chemicals pass through the placenta and enter the foetal environment. Likewise, exposure to these chemicals in the young infant can be assessed by measuring them in maternal samples, including breast milk, as well as measuring them directly in the infant's blood although sensitive techniques must be employed because of strict ethical limits on the amount of blood that can be collected from this age group. On the other hand, the assessment and interpretation of exposures to non-persistent chemicals are difficult for all age groups primarily because by definition, non-persistent chemicals have short half-lives in the body and thus are rapidly eliminated, the intermittent nature of exposures, and sometimes the case of the parent chemical metabolizing in the body to the same chemical as its environmental degradate. The biological half-lives for the non-persistent chemicals can vary from a few minutes for some of the volatile organic chemicals to about 1 day for the organophosphorus insecticide, chlorpyrifos [12]. Therefore, for assessing exposure to such chemicals in adults via biomontioring, recent activities affecting exposures (e.g. eating contaminated foods, use of personal care products) will affect the chemical concentrations in an individual. For example, if the primary route of exposure for a given non-persistent chemical is dietary ingestion, depending on the half-life of the chemical, the blood concentrations rapidly peak and then decrease while the parent chemical/metabolite concentrations in urine rises less rapidly and declines less rapidly than in blood [13]. So assessing exposure to non-persistent chemicals especially for an individual is subject to some exposure classification error because of the effect of activities prior to sample collection and the potential metabolism/degradation issue mentioned. However, in large studies like the National Health and Nutrition Examination Survey (NHANES) [14], because of the large number of participants, the timing of the sample collection relative to an exposure activity is of less concern [3,11]. For assessing *in utero* exposures to non-persistent chemicals, maternal blood and urine samples and cord blood are preferred [11]; however, research efforts are underway utilizing maternal and infant's hair and nails, amniotic fluid and meconium.

These chemical-class biomonitoring examples exemplify that exposure assessment in studies of adverse developmental effects, which may manifest themselves months or years after exposure, are difficult. In retrospective studies, it is much easier to relate effects with exposure to persistent chemicals than for non-persistent chemicals, including for the reasons given above and also because of the potential availability of banked serum samples. The National Children's Study [10] will bank various biological matrices collected at defined times and environmental samples and personal monitoring samples and hence will be able to utilize these banked samples for nested case–control and longitudinal studies. The analyses of these samples coupled with questionnaire information will lead to a more accurate exposure assessment during the critical time window for a particular health outcome. This accurate exposure assessment, which includes other exposure scenario information, and the large number of children enrolled in the National Children's Study should result in a better understanding of the linkage between exposures to environmental chemicals and selected developmental effects.

Exposure assessment at CDC: NHANES selection criteria

Most recently, CDC's biomonitoring efforts have included providing general population exposure assessment data, published in CDC's *National Report on Human Exposure to Environmental Chemicals* [15], and collaborating in epidemiological studies designed to study associations between exposures to environmental chemicals and health outcomes. The *National Report on Human Exposure to Environmental Chemicals* utilizes the analyses of biological samples (blood and its components and urine) to provide an ongoing assessment of the exposure status of the US population to selected environmental chemicals. These chemicals, which are selected based on a nomination process [16], are measured in biological samples collected as part of the NHANES, which dates back to 1971–1974 (NHANES I) although no environmental chemicals were measured in NHANES I. Exposure to lead has been assessed in each of the NHANES beginning with NHANES II (1976–1980), followed by the Hispanic HANES (1982–1984), NHANES III (1988–1994), and since 1999 the continuous NHANES. Lead remains the signature chemical in NHANES, although in NHANES 2003–2004 the number of chemicals measured will have increased to approximately 275. Exposure to many of the chemicals measured in NHANES has been associated with developmental effects.

The number of analytes measured in the continuous NHANES has increased from 27 (in 1999) to approximately 275 for the 2003–2004 *National Report on Human Exposure to Environmental Chemicals*. Most of these measurements are conducted on a random one-third subset of the approximately 10,000 NHANES participants, including pregnant women, sampled from 30 localities over a 2-year period. The NHANES participants are stratified by age, race/ethnicity, and sex and are chosen from the civilian, non-institutionalized US population. The participants represent a national probability sample; thus, the results can be extrapolated to include the entire US population. The minimum age for participation is 2 months, but urine samples are not collected from participants less than 6 years of age. Blood samples for most environmental chemical measurements are collected from participants 12 years and older; however, because of the small amount of blood required, lead, cadmium and mercury are measured in all participants providing a 9-ml blood sample (most of the blood is used for nutritional and clinical testing) and who are at least 1 year of age. For a similar reason, cotinine is measured in everyone 3 years of age and older; they are asked to provide 22 ml of blood. The participants, or their parents, also complete an extensive questionnaire on demographics and health behaviours and undergo a complete physical and medical examination.

Exposure assessment at CDC: NHANES trend data

When sufficient population temporal data have been accumulated on a given chemical, trends in exposure over time can be evaluated. One reason that lead is the signature chemical for NHANES is the relative ease of accurately assessing exposure to lead by measuring its concentrations in small amounts of blood. But the primary reason has been the concern of lead exposure on the developing brain and the resulting effects such as decreases in population intelligence and increases in later delinquent and criminal activities. Blood lead concentration data in children showed initially a precipitous and later a continuous decrease from NHANES II (1976–1980) to NHANES 2001–2002 [17]. This decrease (from 16 μg/dl in 1976 to less than 2 μg/dl in 2001–2002) corresponds with the removal of lead in gasoline in the USA, starting in the mid-1970s; the banning of lead solder in domestic food cans was also enacted during this time period and later the lead abatement programmes in housing were initiated. In many ways, this has been a public health victory. Certainly in examining the exposure effect continuum, lead is a good example of how the enactment of risk management strategies has led to a decrease in blood lead concentrations. If the continuum holds and if the exposure dose: effect curve approaches linearity, obviously we should also be seeing a decrease in effects although there has been concern voiced that the Federal action blood lead level should be decreased from 10 μg/dl to 2 μg/dl [18]. The Federal action blood lead level has decreased in a stepwise fashion from 60 μg/dl beginning in 1960.

Many of the persistent organic chemicals have been implicated as developmental toxicants in animals and to a lesser extent in human beings; however, their measurements take more blood and their developmental effects related to blood concentrations are not as defined as those for lead. Nevertheless, their potential toxic effects are of concern. These persistent organic chemicals include selected chemicals from the following halogenated classes of chemicals: polychlorinated dibenzo-*p*-dioxins; polychlorinated dibenzofurans; polychlorinated biphenyls; organochlorine insecticides; polybrominated diphenylethers (PBDE); and polyfluoroalkyl chemicals (PFC). Twelve of the chlorinated chemicals (or chemical groups) listed in the classes above are the targeted chemicals of the Stockholm Convention on Persistent Organic Pollutants of 2001 [19]. In general, environmental and human concentrations of these chlorinated chemicals have been decreasing in industrialized countries over the last several decades. The primary reasons for this include legislative efforts, which include banning or decreasing of their production and uses and also less emissions from industrial sources. Such legislative bans in the USA date back to the early 1970s for many of the chlorinated chemicals, while voluntary bans in the USA on production of two of the PBDE formulations and the two most prominent PFCs [perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA)] by the sole manufacturer of PFOS and decreased emission by the largest PFOA manufacturer have been enacted in the 21st century. Already, the NHANES data from 1999–2000 to 2003–2004 revealed that the geometric means for PFOS and PFOA have decreased over this time by 32% and 25%, respectively [20]. The PBDEs were measured in individual samples from NHANES for the first time in serum samples collected in 2003–2004 [21]. The PBDEs are similar to the chlorinated chemicals in that they are lipophilic and hence circulate in the body with the lipids in blood and are stored in the adipose tissue. On the other hand, the PFCs are bound to proteins in blood and are involved in enterohepatic circulation and are bound to liver proteins, as well. Therefore, while these halogenated chemicals are all deemed to be persistent in the environment and in people, the PFCs are different in their distribution within the body. Another difference among them is that the primary exposure route for the chlorine containing chemicals is through the human food chain (historically, the dietary ingestion route accounts for about 95% of the general population exposures), while the primary exposure pathways for the PBDEs and PFCs are not fully determined.

The organochlorine chemical with the highest detection rate in CDC's *National Report on Human Exposure to Environmental Chemicals* is 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p′-DDE), which is both an environmental degradate and a metabolite of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (p,p′-DDT), which has been linked to neurological developmental disorders [22]. Although production of DDT was banned in the USA in 1972, serum concentrations of DDE were measured in the 1999–2000 and 2001–2002 NHANES population, aged 12 years and older (Table. 1). Of special

Table 1.
p,p′-DDE (whole weight), geometric mean and selected percentiles of serum concentrations (in ng/g serum or parts per billion) for the US population aged 12 years and older, National Health and Nutrition Examination Survey, 1999–2002 (from Third National Report on Human Exposure to Environmental Chemicals).

	Survey years	Geometric mean (95% confidence interval)	Selected percentiles (95% confidence interval)				Sample size
			50th	75th	90th	95th	
Total, age 12 and older	99–00	1.54 (1.33–1.79)	1.31 (1.09–1.66)	3.49 (2.97–4.27)	7.49 (6.14–9.25)	11.6 (9.25–14.8)	1964
	01–02	1.81 (1.64–2.01)	1.57 (1.37–1.72)	3.97 (3.43–4.59)	8.81 (7.85–10.1)	15.4 (12.9–17.6)	2298
Age group							
12–19 years	99–00	0.561 (0.488–0.646)	0.518 (0.433–0.603)	0.872 (0.682–1.18)	1.52 (1.13–2.25)	2.31 (1.76–3.56)	686
	01–02	0.623 (0.534–0.726)	0.592 (0.495–0.727)	0.997 (0.819–1.22)	1.65 (1.39–2.07)	2.30 (1.91–3.14)	758
20 years and older	99–00	1.83 (1.56–2.14)	1.61 (1.26–2.07)	4.17 (3.48–4.66)	8.12 (6.37–10.6)	12.3 (9.87–16.7)	1278
	01–02	2.14 (1.91–2.39)	1.76 (1.61–2.04)	4.59 (4.10–5.26)	9.75 (8.34–11.5)	16.8 (13.7–19.1)	1540
Gender							
Males	99–00	1.49 (1.30–1.70)	1.25 (1.10–1.44)	3.01 (2.56–3.74)	6.43 (5.40–8.00)	9.63 (6.63–15.6)	937
	01–02	1.77 (1.57–2.01)	1.59 (1.36–1.76)	3.40 (3.03–4.10)	7.48 (6.43–8.75)	13.1 (9.66–17.6)	1069
Females	99–00	1.59 (1.32–1.92)	1.38 (1.03–1.99)	4.05 (3.15–4.79)	8.12 (6.36–11.5)	13.2 (9.81–18.5)	1027
	01–02	1.85 (1.66–2.06)	1.49 (1.32–1.75)	4.57 (3.81–5.47)	10.1 (9.01–11.9)	16.8 (13.4–19.7)	1229
Race/ethnicity							
Mexican-Americans	99–00	3.92 (3.40–4.51)	3.52 (3.17–3.91)	8.20 (7.26–10.4)	22.0 (12.2–32.2)	31.5 (19.7–48.1)	657
	01–02	3.92 (3.37–4.57)	3.53 (2.68–4.34)	9.33 (7.31–12.5)	26.6 (17.9–38.3)	40.9 (26.8–90.5)	566
Non-Hispanic blacks	99–00	1.63 (1.31–2.02)	1.34 (1.11–1.66)	3.80 (3.01–5.69)	11.1 (6.57–13.2)	14.6 (8.88–35.2)	416
	01–02	1.82 (1.46–2.28)	1.38 (1.22–1.72)	4.39 (3.52–6.06)	10.5 (7.24–17.6)	19.3 (8.51–49.3)	515
Non-Hispanic whites	99–00	1.32 (1.14–1.53)	1.13 (1.01–1.35)	2.85 (2.34–3.36)	5.71 (4.62–6.53)	8.04 (6.32–9.81)	732
	01–02	1.57 (1.39–1.76)	1.41 (1.27–1.58)	3.10 (2.56–3.68)	7.00 (6.02–8.34)	11.3 (8.60–13.7)	1053

note is that DDE is detectable in the younger population even though they were born years after US use and production of DDT ceased. Furthermore, of interest is that the Mexican-American population had DDE serum concentrations two to three times higher than non-Hispanic whites or non-Hispanic blacks. The age breakdown of adults is not evident in fig. 3, but we have analysed serum pools made from serum collected from NHANES 2001–2002 participants and examined the concentrations for these halogenated chemicals, including DDE (fig. 3) [23]. There are several apparent observations regarding DDE concentrations in the various demographic groups: (i) Mexican-Americans have the highest 50th and 95th percentile concentrations; (ii) except for the Mexican-Americans these concentrations increase with age; and (ii) as the population ages, DDE concentrations become higher in females than in males. (i) A possible explanation for higher concentrations in the Mexican-American population is that they were exposed to higher environmental DDT/DDE concentrations while possibly living in Mexico or by eating greater quantities of DDE-contaminated food. (ii) An explanation for the concentrations increasing with age is that within the USA the older population was exposed to environmental concentrations of DDE during the time when its environmental concentrations were higher than they are now and also DDE has a long biological half-life so that the body retains much of the DDE over time; however, the highest concentrations of DDE being found in younger Mexican-Americans is probably due to continued exposure. (iii) An explanation for DDE concentrations being higher in older females compared to older males is not as readily apparent, although it is probably an issue of pharmacokinetics, such as elimination rates, and not exposure because younger males have higher concentrations than younger females (i.e. higher exposures). A similar sex difference is also seen for dioxin and dioxin-like chemicals as determined by their dioxin toxic equivalents but tends to be opposite for the PBDEs and PFCs in the same serum pools [24]; that is, their concentrations in older populations are higher in males. As mentioned, the temporal exposure scenario to these chemicals differ: for the chlorinated chemicals, the highest exposures ceased decades ago so we are now on the back end of the serum concentration decay curve of

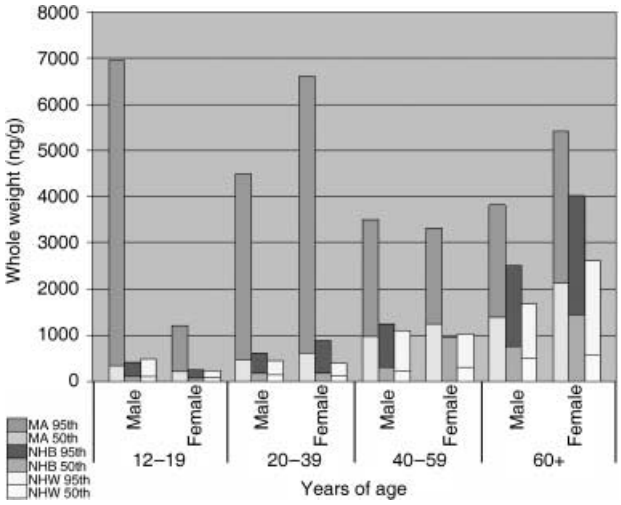

Fig. 3. The 50th and 95th percentile of DDE by age group, race and sex as measured in serum pools from NHANES 2001–2002.

these chemicals in the US population, while for the PFCs and PBDEs in 2001–2002 we were still on the highly exposed portion of the curve. In general, for all of these halogenated chemicals, the younger male population has higher concentrations than the equivalent younger female population. This is consistent with a scenario of higher exposures in the males and also with scenarios when exposures dominate relative to pharmacokinetics, males have higher concentrations, but when pharmacokinetics dominate (e.g. when high exposures have ceased and half-life is the primary factor on serum concentrations), older females generally have higher concentrations than older males. As concentrations of several congeners of PFOS, PFOA and several congeners of PBDEs decrease in human beings over the next several years, it will be interesting to note any changes in the relative concentrations, with regard to age and sex. In addition to the samples collected in the USA, we have also measured the PFCs in serum from Faroe Island children (collected in 1993–1994 and 2005) and pregnant women (collected in 2000) and from men and pregnant women in Peru [25]. The samples from the Faroe Islands and from the USA showed similar concentrations when sampling periods were similar; on the other hand, the samples from Peru were mostly non-detectable for both PFOS and PFOA. Although the numbers of samples analysed from the Faroe Islands were not high, of interest is that the median concentration for PFOS and PFOA decreased in the 7-year-old children sampled in 1993–1994 (n = 103) from 26.3 p.p.b. and 5.0 p.p.b., respectively, to 16.3 p.p.b. and 4.5 p.p.b. in 5-year-old (n = 12) sampled in 2005. This is consistent with the 3M Company discontinuing its production of PFOS in early 2000s.

Foetal exposures to organophosphorus insecticides have also been linked to developmental effects, including reduced birth length and weight and reduced head circumference [26–28]. These effects have been shown not only to be linked with organophosphorus exposure and the variability of the paroxonase 1 (PON1) gene, which has several nucleotide polymorphisms. These various forms of the PON1 gene signal the body to make the PON1 enzyme that in essence detoxifies the organophosphorus insecticide. The PON1 enzyme differs among people both in terms of concentration and activity [29]. Although exposure to these chemicals can be intermittent, a low level chronic exposure from dietary residues likely exists. In CDC's *National Exposure Report*, the population levels presented likely represent this dietary exposure as the large sample size probably minimizes the contribution of 'spikes' in exposure from intermittent dermal, inhalation or non-dietary ingestion routes of exposure. As a part of the Food Quality Protection Act of 1996 [30], the US Environmental Protection Agency (EPA) was tasked with re-evaluating all permissible food tolerances and to consider both aggregate and cumulative exposures from chemicals with the same mode of action. Because the organophosphorus insecticides share a common mode of action as cholinesterase inhibitors, they were among the first class of pesticides whose tolerances were re-evaluated. As a result of this re-evaluation, food tolerances of the most commonly applied organophosphorus insecticide, chlorpyrifos, were reduced by a factor of three. In addition, all US residential applications of the organophosphorus insecticides chlorpyrifos and diazinon were legislatively banned at the end of 2001 and 2002, respectively. Thus, we were able to use the NHANES measurements as a tool to evaluate the efficacy of these regulatory actions in reducing exposures to organophosphorus insecticides.

Both selective and non-selective metabolites of organophosphorus insecticides were measured as a part of the continuing NHANES. Data generated from population-based samples collected in 1999 and 2000 indicated that a large proportion of the population was exposed to the organophosphorus insecticide chlorpyrifos and other diethyl substituted organophosphorus insecticides or their environmental degradates. Data obtained from subsequent NHANES collections revealed that the frequency of detection of these organophosphorus metabolites and the median concentrations of these organophosphorus metabolites have decreased almost 2-fold following the risk management procedures [31]. Thus, these data indicate that the regulatory actions undertaken by the EPA have indeed resulted in a reduction in exposure to the US population.

In addition, these organophosphorus insecticide metabolite data can be used in conjunction with known pharmacokinetic data and environmental degradation data to conduct some backwards dosimetry estimates of exposure. For example, data from 1999 to 2000 for 3,5,6-trichloro-2-pyridinol, the primary selective metabolite of chlorypyrifos, can be used to estimate population exposure to chlorpyrifos that can then be compared to reference values such as the EPA's reference dose or population-adjusted dose (PAD) or the Food and Drug Administration's Allowable Daily Intake dose [31]. For 1999–2000, the backwards dosimetry-estimated doses are lower than the chronic PAD for children; however, the 95th percentile dose was within a factor of 2 of the chronic PAD [31]. These data indicate that most children aged 6–11 years are receiving dietary doses below those set as a permissible dose by the EPA; however, the most highly exposed children are exposed at levels close to this established set point.

Exposure assessment based on age of the population

The relation between age of the population and biomonitoring concentrations are relevant to developmental effects. As mentioned above, for the traditional chlorinated persistent organic chemicals and for polybrominated biphenyls, for which exposure to chemicals in both classes started declining years ago, the older members of the general population have in general the highest body burden levels. However, that is not true for the persistent classes of chemicals that are in current use. For example, in the NHANES 2003–2004 data, we found that in general for PBDEs age (age 12 years was the youngest age sampled) was inversely related to serum concentrations PBDEs [21], and that there was no significant trend with PFCs and age although the youngest population (again beginning at age 12 years) tended to have the highest

serum concentrations [20]. In concordance with this, the NHANES 1999–2002 data showed that blood lead levels were the highest in the 1–5-year-old age group. Similarly, serum cotinine concentrations were the highest in the youngest age range examined, the 3–11-year-old age range. This same trend continued for several other classes of environmental chemicals measured in ages 6 years and older including the organophosphorus pesticides, both as the non-specific dialkyl phosphate metabolites and as their specific metabolites; several phthalates; and 1,4-dichlorobenzene, as determined by its metabolite, 2,5-dichlorophenol. We know that children, proportionate to their weight and surface area, consume more water, food and air (including inhaling air from a lower breathing zone) than adults [32]. Hence, when these pathways are involved in exposure to environmental chemicals, this implies that children may have higher exposures. In addition, the pathways of exposure differ in children (and change with age of child) [13] as their daily habits such as mouthing many objects, crawling on floor and playing in soil change with age. These higher levels of environmental chemicals found in children have been noted previously for lead [33,34], pesticides [35] and even when recent exposures to a persistent chlorinated organic chemical 2,3,7,8-tetrachlorodibenzo-p-dioxin were evaluated in the Seveso incident in 1976 [36]. These higher levels in the youngest age groups monitored could be because of relative increases in exposure but also in differences in pharmacokinetics (absorption, distribution, metabolism and elimination); in short, how children handle certain chemicals compared to adults. For example, following exposure to lead, children have a higher absorption rate and also as children grow, their bones of course grow and thus stores more lead; hence, young children have higher blood lead levels, even assuming equal exposure, because of increased absorption and increased bone storage. When examining the issue of higher concentrations of environmental chemicals in children, one also has to examine the effect of breast feeding. One example of that is a study of children in the Faroe Islands potentially exposed to PCBs [37]. We found that the serum concentrations of higher chlorinated PCB congeners within an individual at age 7 years and 14 years were highly correlated, but the concentrations tended to be two to three times higher in the 7-year-old. Similarly, the umbilical cord PCB concentrations, which are predictors of the pregnant woman's concentrations, were also highly correlated but similar in concentrations to that of the 14-year-old age group, reflecting similar diets. Of interest is that the duration of breastfeeding and whale blubber consumption were significant predictors of total PCB concentrations in the population both at 7 and 14 years of age.

This segues directly in the questions regarding concentrations of environmental chemicals in women of reproductive age and in women throughout pregnancy. Very little information is available on changes in the concentrations of environmental chemicals in women during pregnancy. One study [38] found that the serum concentrations of selected organochlorine insecticides did not change significantly when the concentrations were adjusted for the change in lipids. We are now analysing the NHANES data to assess differences in concentrations of a variety of environmental chemicals in serum collected during the three trimesters.

Additional approaches: broad scan analysis and biomarkers of effect

Thus far, we have discussed linking adverse developmental outcomes with concentrations of targeted chemicals, which is akin to 'only looking under the lamppost'. Another approach that has been underutilized is broad scan analysis, which could be applied to case–control studies. This approach involves analysing a set of cases with the disease outcome and a set of controls. The analytical approach involves as little sample preparation work as possible, so as not to 'lose' any exogenous or endogenous chemicals of potential interest, and then injecting the biological matrix or its extract into a high-resolution chromatographic system that is coupled to a universal type of detector, such as time of flight mass spectrometer – which provides a full scan mass spectrum of all eluting chemicals. Then, computer-assisted statistical techniques such as principal component analysis are used to differentiate the chemicals for example that are common to the cases but not to the controls. The focus of course would then shift to this set of chemicals. Such techniques will no doubt be used more in the future when attempting to understand disease states believed to be linked with environmental chemicals but with uncertain aetiology.

Both the targeted analysis approach and the broad scan analysis approach measure biomarkers of exposure and attempt to assess exposure and then link that exposure to effects. Another approach that has also been underutilized is to measure biomarkers of effect and then work either backwards on the exposure effect continuum to link with exposures or forward to link effect markers with clinically observed effects. Such approaches are being used in the 'omics' techniques.

Conclusions

Exposure assessment for determining the impact of exposure to environmental chemicals with adverse developmental outcomes is quite contentious. Indirect techniques, primarily questionnaire information, have been used historically to estimate the degree and frequency of exposure. Today, the emphasis is shifting to the analytical measurements of environmental chemicals but particularly to those measurements of biological samples (biomonitoring). While biomonitoring provides direct information as to what chemicals and how much are being absorbed into the body, we have to realize that it is generally a one-time snapshot of an individual's exposure. The accuracy of this snapshot depends on the chemical of interest, the life stage of the population, the availability and proper collection of the biological sample, and of course the analytical method itself. Furthermore, biomonitoring is in general quite expensive, and for this and for other reasons involving the uses of biomonitoring data,

more accurate modelling techniques for interpreting biomonitoring data are being developed. Biomonitoring techniques, although potentially quite powerful, are but one tool used to picture the exposure scenario. These data need to be coupled with other information such as pharmacokinetic data, genetic information and demographic information. Certainly, the development of background biomonitoring information as exemplified here will be of assistance. However, we still lack sufficient information on exposures to many chemicals of interest during pregnancy and the foetal and early childhood periods. Certainly, analytical approaches differ for assessing exposures during these various life stages [39]. Most of our attention is being focused on targeted analysis in a laboratory setting, which involves the pre-selection of the chemicals of interest. In the future, more efforts will be expended to real-time field measurements of targeted chemicals but also to the broad scan approach for elucidating exposure differences in case populations and controls. Attention will also be directed towards measuring subclinical biomarkers of effect. It is also important to note that exposure assessment cannot be done in a vacuum. Relevant information is needed from other disciplines, including toxicologists, epidemiologists, modelers and public health officials. Through the use of such a network, biomonitoring data will continue to increase in use in risk assessment and risk management decisions and ultimately in policy and legislation.

References

1 National Academy of Sciences. Committee on National Monitoring of Human Tissues. Monitoring Human Tissues for Toxic Substances. National Academy Press, Washington, DC, 1991.

2 Angerer J, Bird MG, Burke T et al. Meeting report: strategic biomonitoring initiatives: moving the science forward. Toxicol Sci 2006;**93**:3–10.

3 Needham LL, Barr DB, Calafat AM. Characterizing children's exposures: beyond NHANES. Neurotoxicology 2005;**26**:547–53.

4 Needham LL, Calafat AM, Barr DB. Uses and issues of biomonitoring. Int J Hyg Environ Health 2007;**210**:229–38.

5 Lanphear BP, Hornung R, Khoury J et al. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect 2005;**113**:894–9.

6 Chen A, Dietrich KN, Ware JH, Radcliffe J, Rogan WJ. IQ and blood lead from 2 to 7 years of age: are the effects in older children the residual of high blood lead concentrations in 2-year-olds? Environ Health Perspect 2005;**113**:597–601.

7 Whyatt RM, Rauh V, Barr DB et al. Prenatal insecticide exposures, birth weight and length among an urban minority cohort. Environ Health Perspect 2004;**112**:1125–32.

8 Aronson M, Hagberg B, Gillberg C. Attention deficits and autistic spectrum problems in children exposed to alcohol during gestation: a follow-up study. Dev Med Child Neurol 1997;**39**:583–7.

9 Strömland K, Nordin V, Miller M, Åkerström B, Gillberg C. Autism in thalidomide embryopathy: a population study. Dev Med Child Neurol 1994;**36**:351–6.

10 The National Children's Study. National Institutes of Health, Rockville, MD. Available from: http://www.nationalchildrensstudy.gov/ [updated 19 July 2007; cited 26 July 2007].

11 Barr DB, Wang RY, Needham LL. Biological monitoring of exposure to environmental chemicals throughout the life stages: requirements and issues for consideration for the National Children's Study. Environ Health Perspect 2005;**113**:1083–91.

12 Nolan RJ, Rick DL, Freshour NL, Saunders JH. Chlorpyrifos: pharmacokinetics in human volunteers. Toxicol Appl Pharmacol 1984;**73**:8–15.

13 Needham LL, Sexton K. Assessing children's exposure to hazardous environmental chemicals: an overview of selected research challenges and complexities. J Expo Anal Environ Epidemiol 2000;**10**:611–29.

14 National Health and Nutrition Examination Survey (NHANES). National Center for Health Statistics, Hyattsville, MD. Available from: http://www.cdc.gov/nhanes [updated 19 July 2007; cited 26 July 2007].

15 National Report on Human Exposure to Environmental Chemicals. Centers for Disease Control and Prevention (CDC), Atlanta, GA. Available from: http://www.cdc.gov/exposurereport [updated 20 March 2007; cited 26 July 2007].

16 Federal R. Final selection criteria and solicitation of nominations for chemicals or categories of environmental chemicals for analytic development and inclusion in future releases of the National Report on Human Exposure to Environmental Chemicals. Notice 2002;**67**:12996.

17 Needham LL, Barr DB, Caudill SP et al. Concentrations of environmental chemicals associated with neurodevelopmental effects in US population. Neurotoxicology 2005;**26**:531–45.

18 Gilbert SG, Weiss B. A rationale for lowering the blood lead action level from 10 to 2 microg/dl. Neurotoxicology 2006;**27**:693–701.

19 Lallas PL. The Stockholm convention on persistent organic pollutants. Am J Int Law 2001;**95**:692–708.

20 Calafat AM, Wong LY, Kuklenyik Z, Reidy JA, Needham LL. Polyfluoroalkyl chemicals in the US population: data from the National Health and Nutrition Examination Survey (NHANES) 2003–2004 and comparisons to NHANES 1999–2000. Environ Health Perspect 2007;**11**:1596–1602.

21 Sjödin A, Wong L-Y, Jones R et al. Serum concentrations of polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyl (PBB) in the United States population: 2003–2004. Environ Sci Technol 2007; doi: 10.1021/es702451p.

22 Eriksson P, Archer T, Fredriksson A. Altered behaviour in adult mice exposed to a single low dose of DDT and its fatty acid conjugate as neonates. Brain Res 1990;**514**:141–2.

23 Needham LL, Patterson DG Jr, Calafat AM, Sjödin A, Turner WE, Kuklenyik Z. Distribution of halogenated environmental chemicals among people of different ages, races and sexes in the United States. Organohalogen Compounds 2006;**68**:484–7.

24 Calafat AM, Kuklenyik Z, Caudill SP, Reidy JA, Needham LL. Perfluorochemicals in pooled serum samples from United States residents in 2001 and 2002. Environ Sci Technol 2006;**40**:2128–34.

25 Calafat AM, Needham LL, Kuklenyik Z et al. Perfluorinated chemicals in selected residents of the American continent. Chemosphere 2006;**63**:490–6.

26 Needham LL. Assessing exposure to organophosphorus pesticides by biomonitoring in epidemiologic studies of birth outcomes. Environ Health Perspect 2005;**113**:494–8.

27 Berkowitz GS, Obel J, Deych E et al. Exposure to indoor pesticides during pregnancy in a multiethnic urban cohort. Environ Health Perspect 2003;**111**:79–84.

28 Eskenazi B, Harley K, Bradman A et al. Association of in uteroorganophosphate pesticide exposure and fetal growth and length of gestation in an agricultural population. Environ Health Perspect 2004;**112**:1116–24.

29 Furlong CE, Cole TB, Jarvik GP et al. Role of paraoxonase (PON1) status in pesticide sensitivity: genetic and temporal determinants. Neurotoxicology 2005;**26**:654–9.

30 Food Quality Protection Act (FQPA) of 1996. Environmental Protection Agency (EPA), Arlington, VA. Available from: http://

www.epa.gov/pesticides/regulating/laws/fqpa/ [updated 28 June 2007; cited 26 July 2007].
31 Barr DB, Allen R, Olsson A et al. Concentrations of selective metabolites of organophosphorus pesticides in the United States population. Environ Res 2005;**99**:314–26.
32 National Research Council Committee on Pesticides in the Diets of Infants and Children. Pesticides in the Diets of Infants and Children. National Academy Press, Washington, DC, 1993.
33 Brody DJ, Pirkle JL, Kramer RA et al. Blood lead levels in the US population. Phase 1 of the Third National Health and Nutrition Examination Survey (NHANES III, 1988 to 1991). J Am Med Assoc 1994;**272**:277–83.
34 Pirkle JL, Kaufmann RB, Brody DJ, Hickman T, Gunter EW, Paschal DC. Exposure of the US population to lead, 1991–1994. Environ Health Perspect 1998;**106**:745–50.
35 Adgate JL, Barr DB, Clayton CA et al. Measurement of children's exposure to pesticides: analysis of urinary metabolite levels in a probability-based sample. Environ Health Perspect 2001;**109**:583–90.
36 Eskenazi B, Mocarelli P, Warner M et al. Relationship of serum TCDD concentrations and age at exposure of female residents of Seveso, Italy. Environ Health Perspect 2004;**112**:22–7.
37 Barr DB, Weihe P, Davis MD, Needham LL, Grandjean P. Serum Polychlorinated biphenyl and organochlorine insecticide concentrations in a Faroese birth cohort. Chemosphere 2006;**62**:1167–82.
38 Longnecker MP, Klebanoff MA, Gladen BC, Berendes HW. Serial levels of serum organochlorines during pregnancy and postpartum. Arch Environ Health 1999;**54**:110–4.
39 Needham LL, Özkaynak H, Whyatt RM et al. Exposure assessment in the National Children's Study: introduction. Environ Health Perspect 2005;**113**:1076–82.

