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The Potential of Exposure Biomarkers in Epidemiologic Studies of Reproductive Health

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To further the development and application of exposure markers in field investigations in reproductive epidemiology, we have synthesized recent examinations of the issues surrounding exposure measurements in reproductive epidemiology. The specific goals of this paper are to define exposure biomarkers and explore their potential uses, particularly as screening tools. The tests for glucaric acid, thioethers, mutagenicity, and porphyrin patterns meet the general criteria for useful exposure screens. For certain xenobiotic agents, these tests accurately differentiate exposure levels, as demonstrated in occupational and environmental epidemiologic studies. As urinary screens, they are noninvasive and applicable on a large scale with current laboratory techniques. For short-term exposure, glucaric acid, thioethers, and mutagenicity tests are useful. Porphyrin patterns may measure cumulative effects as well as current exposure levels. The usefulness of these tests in epidemiologic studies of environmental effects on reproductive health has yet to be studied. To do so, the battery must be standardized for pregnant women, and test results must be correlated with measured adverse reproductive outcomes, such as gestational length and birthweight. This correlation is particularly important because maternal exposure rather than fetal exposure is being measured. The extent to which xenobiotic chemicals cross the placental barrier may vary greatly depending on the type of exposures, timing in pregnancy, and maternal detoxification capability. Without better exposure measures, epidemiologic studies of reproductive health probably will not successfully identify xenobiotic fetotoxic agents in the environment. However, with an adequate battery of nonspecific exposure biomarkers, prospective studies of environmental effects on pregnancy outcomes might be possible. To narrow the list of potential exposures, these prospective studies could be followed by case-control studies of more specific biomarkers directed at suspect exposures.

Introduction

The research of Hatch (*1*) has greatly increased our understanding of the use and potential limitations of biological markers for adverse reproductive effects. Likewise, there are great potentials and pitfalls in the emerging field of exposure biomarkers for reproductive health. This area of research has lagged considerably behind the field of developing new effect markers, such as semen analysis and early pregnancy loss. However, its importance in quantitative risk assessment cannot be overemphasized. As Hulka has so clearly stated, "the most important current limitation in many epidemiological studies is the relative inaccuracy of methods for measuring the exposure variable" (*2*).

Exposure to a potential fetal health hazard can be estimated through ecological assessment (e.g., testing the community water supply), questionnaires (e.g., classifying residents according to whether they drink bottled or tap water), or biological markers (e.g., testing for exposures to chemicals or solvents found in tap water). In a community with an environmental factor suspected of adversely affecting reproductive outcome, measuring environmental contaminants provides a gross, ecological estimate of the exposure incurred by pregnant women and their fetuses. However, ecological estimates can lead to significant misclassification of individual exposure (*3*). Such misclassification, if nondifferential, will underestimate the true effect of the exposure. If misclassification is differential, misleading results in either direction can occur.

Using questionnaires to assess the extent of an individual's potential for exposure may help to reduce misclassification bias. Yet reports of individual exposures can be erroneous in either direction (*4*). In addition, people are often unaware of their potential for exposure, and researchers may not know or be able to account for all the pathways of exposure. For example, certain mothers in a Yugoslav community with a lead smelter had elevated blood-lead levels. Questionnaire data determined which of these women were wives of men employed in the lead industry. However, these data could not distinguish between women with low blood-lead levels and women with elevated levels (*4*). In this example, a biological exposure marker (blood lead-level) was available for classifying mothers according to their exposure to lead. To date, such biological markers have not been widely available nor have they been widely used when they are available.

Several years ago, the Environmental Protection Agency cosponsored a National Research Council study on The Role of Biomarkers in Reproductive and Developmental Toxicology (*5*).

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After reviewing the situation, Longo described a "paradox" (*6*). Although several techniques for identifying individual exposures have been developed and tested, and although more and more xenobiotics have been recognized to have teratogenic and mutagenic potential, "essentially no specific biomarkers are currently available to indicate that exposure to a given xenobiotic is directly associated with a cellular, subcellular, or pharmacodynamic event" (*6*). The paradox continues, despite continuing advancements in laboratory science and the growing recognition of the need for biological markers to improve exposure measurement in the field of environmental epidemiology (*3,4,7–9*). To further the development and application of exposure markers in studying the environmental hazards to reproductive health, we have attempted to synthesize recent examinations of the issues surrounding exposure measurements in reproductive epidemiology. The specific goals of this paper are to explore the potential uses of biomarkers as measures of exposure, particularly as they may be used in an environmental setting as screening tools.

Biomarkers As Measures of Exposure

Hulka defines biological markers in environmental epidemiology as "cellular, biochemical or molecular alterations which are measurable in biological media such as human tissues, cells or fluids and are indicative of exposure to environmental chemicals" (*2*). Biomarkers are not environmental measures in air, soil, water, or food; nor reports from research subjects; nor results of physical, anthropometric, or mental examinations. Rather, they are material measures obtained from physical samples (*4*).

Biomarkers used to estimate environmental exposures must be distinguished from those used to estimate the effects of those exposures. Biological markers of effects can be subclassified into biologically effective doses (such as DNA adducts) and biological responses (*2*). Examples of the biological response include sensitive tests of early pregnancy loss and serum alphafetoprotein to detect fetal neural tube defects. The National Research Council study primarily dealt with effect markers (*5*). Although both exposure and outcome measures are necessary for an epidemiologic investigation, we will focus on measuring exposures. In Hulka's classification, these are "internal dose markers" (*2*).

Internal dose-exposure markers may be useful to improve the quality of exposure measurement in an epidemiologic investigation of a known environmental hazard; to serve as the "gold standard" for other information sources; to provide a screening tool for environmental exposures to a target tissue (in this case, the fetus); and to provide quantification of the biological load from a known exposure (*4*). To be useful in epidemiologic investigations of reproductive health an exposure marker should be better than the woman's ability to recall an exposure; allow for differentiation between exposure levels, at least qualitatively; allow the use of noninvasive procedures that are applicable on a large scale; and provide interpretable data for short-term or cumulative exposure regarding time, dose, and duration (*4*).

For the environmental Sherlock Holmes, internal dose markers offer strong circumstantial evidence that the perpetrator xenobiotic has invaded the human victim. This evidence is very specific if the chemical is retrieved unaltered. However, substantial circumstantial evidence can be gleaned from metabolically altered chemicals. The metabolic outcome can be very specific (e.g., urinary cotinine for nicotine in cigarette smoke) or nonspecific (e.g., thioethers for cigarette smoking). Nonspecific markers measure a biochemical pathway affected by a variety of xenobiotic agents.

Exposure Markers As Screening Tools

In addition to possessing the characteristics of all useful exposure markers, biomarkers used as exposure screens should be able to detect subtoxic exposures and be nonspecific (*8*). Nonspecificity of the marker is important because the environment commonly includes complex and unknown chemical mixtures, such as those found in drinking water, that could be misclassified by selecting a few specific markers for a screening battery.

For epidemiologic research, nonspecific markers tend to be held in lower esteem than specific markers, since it is impossible, without further evidence, to identify which chemical has triggered the metabolic response being measured. However, as screening tools, nonspecific markers hold some promise. A biomarker that can be used to detect that one or more of a class of xenobiotic agents to which the pregnant woman has been exposed and may have exposed her fetus could be useful for targeting a subset of women for further investigation and follow-up. First, however, the fact that the metabolic pathway has been altered must be correlated with adverse human reproductive outcomes so that such alteration can be shown to reflect fetotoxicity.

We have previously proposed three nonspecific urinary biomarkers as potential screening tools for reproductive epidemiology (*8*): glucaric acid, thioethers, and porphyrin patterns. Vainio et al. (*10*) proposed mutagenic activity in bacteria as another nonspecific urinary screening tool, and Brewster (*9*) has added that biomarker to her proposed battery for pregnant women. Let us briefly review the usefulness of these biomarkers.

Bacterial Urinary Assay for Mutagenic Activity

Vainio et al. (*10*) critically reviewed the bacterial bioassay procedure as a test of mutagenic activity in urine. Like the other tests proposed, it has the advantage of demonstrating biological activity rather than the mere presence of xenobiotic substances. However, this procedure poses problems that lead the reviewers to recommend that it be used in conjunction with other screening measures. We agree. For example, a bacterial bioassay cannot detect cumulative exposures and may react to substances normally present in urine (such as amino acids). Table 1 gives examples of the numerous studies of reported alterations in mutagenicity by external agents (*11–59*). Occupational exposures have been associated with alterations in mutagenicity; however, not all studies are positive. For example, oncology nurses handling cytotoxic drugs have been studied by several investigators. Two studies were positive for altered mutagenicity (*13,17*), but two later reports were negative (*25,42*). This difference may reflect changes in routines for handling these drugs.

Table 1. Reported alterations in mutagenicity by xenobiotics.

Urine[a]	Human studies	References
+	Anesthesiologists (halogenated anesthetic gases)	McCoy et al. (*11*)
+	Epichlorhydrin-exposed workers	Kilian et al. (*12*)
+	Oncology nurses (cytotoxic drugs)	Falck et al. (*13*)
+	Foundry workers (PAHs)	Schimberg et al. (*14*)
+	Refinery workers (coal tar and pitch)	Inamasu et al. (*15*)
+	Chemical workers (various)	Dolera et al. (*16*)
+	Oncology nurses (cytotoxic drugs)	Bos et al. (*17*)
+	Pharmacists (cytotoxic drugs)	Anderson (*18*)
+	Pharmacists (cytotoxic drugs)	Nguyan et al. (*19*)
		Theiss (*20*)
+	Carbon electrode industry workers	Pasquinin et al. (*21*)
+	Chemical and coke oven workers	Kriebel et al. (*22*)
+	Cold—rolling steel plant workers (mineral oils); synergistic with smoking	Pasquini et al. (*23*)
+	Vegan diet	Sasson et al. (*24*)
+	Cancer patients on chemotherapy	Benhamou et al. (*25*)
+	Workers at a coal tar distillation plant	Jongeneelen et al. (*26*)
+	Workers at sewage treatment plants	Scarlett-Krans et al. (*27*)
+	Tool and die workers (*N*-nitroso diethanolamine, diethylnitrosamine)	Garry et al. (*28*)
+	Cancer patients receiving cyclophosphamide (or doxorubicin)	Tuffnall et al. (*29*)
+	Fried salmon diet to nonsmokers (reversed by co-intake of parsley)	Ohyama et al. (*30*)
?	Tannery workers	Gostantini et al. (*31*)
+	Steel mill workers, coal processing	DeMeo et al. (*32*)
+	Explosives manufacturing workers (trinitrotoluene)	Ahlborg et al. (*33*)
+	Smoking, passive	Mohtashamipur et al. (*34*)
+	Smoking >25 years	Kriebel et al. (*35*)
+	Smoking	Curval et al. (*36*)
−	Snuff users	Curvall et al. (*36*)
±	Pharmacists handling cytostatic drugs	Kolmodin-Hedman et al. (*37*)
−	Coke oven emissions exposure	Moller and Dybing (*38*)
−	Tire plant workers	Falck et al. (*39,40*)
−	Tire plant workers (possible smoking synergism); tetramethyl (thiuram disulfide, poly-*p*-dinitrosobenzene, diaryl-*p*-phenylendiamines)	Grebelli et al. (*41*)
−	Oncology nurses handling cytotoxic drugs	Barale et al. (*42*)
−	Anode plant workers exposed to coal tar and pitch	Venier et al. (*43*)
−	Oncology nurses handling cytostatic drugs	Benhamou et al. (*25*)
−	Autopsy service workers (formaldehyde)	Connor et al. (*44*)
−	Nonsmokers on low PAH diet exposed passively to tobacco smoke	Scherer et al. (*45*)
−	Hospital employees handling cancer chemotherapy agents	Everson et al. (*46*)
		Staiano et al. (*47*)
−	Macrobiotic versus typical western diet	Sasson et al. (*24*)
−	Workers exposed to 2,4,7-trinitro- 9-fluorenone (low dose)	Grebelli et al. (*48*)
−	Operating room personnel	Baden et al. (*49*)
Animal studies		
+	Acrylonitrile	Mori et al. (*50*)
+	Food mutagens (quercetin and rutin)	Grebelli et al. (*51*)
+	Coal tar, dermal	Jongeneelen et al. (*26*)
+	Hazardous industrial waste samples	DeMarini et al. (*52*)
+	2,4,7-Trinitro-9-fluorenone	Crebelli et al. (*48*)
+	Benzo(*a*)pyrene	Jongeneelen et al. (*53*)
+	Cyclophosphamide	Duverger-van Bogaert et al. (*54*)
+	Azo dye tartrazine	Henschler and Wild (*55*)
Amniotic fluid		
+	Smoking (heavy) versus nonsmoking, at term	Rivrud et al. (*56*)
−	Smoking versus nonsmoking, 2nd trimester	Everson et al. (*58*)
		Rivrud et al. (*57*)
Direct chemical mutagenicity		
	Compilation of results of >5000 chemicals	EMIC Index (*58*)

[a](+) Indicates a slight increase of questionable significance.

Table 2. Reported alteration in D-glucaric acid excretion by xenobiotics.

Excretion[a]	Study	Reference
↑	Pesticide production workers (aldrin, dieldrin, endrin, DDT)	Hunter et al. (65)
↑	Endrin manufacturing plant workers	Notten and Henderson (60) Ottervanger and Van Sitter (66) Vrij-Standhardt et al. (67)
↑ (In some)	Metal and chemical factory employees	Notten and Henderson (60)
↑	Pesticide packaging workers	Seutter-Berlage et al. (68)
↑	Electrical workers exposed to PCBs	Seutter-Berlage et al. (69)
→	Workers in polyester industry (styrene)	Hotz et al. (70)
→	Resident of Times Beach, Missouri, in high-exposure risk group for dioxin	Steinberg et al. (71)
→	Steel plant employees (low-risk exposure to mineral oils)	Pasquini et al. (23)
↑	Diuroin, hexachlorobenzene, heptaclor, dieldrin, aldrin, rhotane, disulfiram, 2-phenylphenol in guinea pigs	Notten and Henderson (61)
±↑	Toluene, tetraethyl lead, Aroclor 1260, ethanol, *n*-hexane, dodin, atrazine in guinea pigs	Notten and Henderson (61)
→	Captan, dimethoate, nitrobenzene, aniline, naphthylacetic acid, benzene, rotenone, binapacryl in guinea pigs	Notten and Henderson (61)

[a](→) Unchanged; (±) slight elevation of questionable significance.

Glucaric Acid

Notten and Henderson (*60*) first proposed using urinary glucaric acid to screen for xenobiotic exposures. Glucuronidation is a major transformation route for a number of xenobiotics, including azides, nitrites, alkylamines, and alkyl and aryl alcohols (*61*). Stimulation of the glucuronidation pathway results in increased excretion of glucaric acid, primarily through reduced production of glycogen and possibly through direct induction of pathway enzymes (*9,62*).

Although pregnancy (*63*) and estrogen therapy (*64*) produce a nonsignificant increase in glucaric acid excretion, a number of exogenous chemicals have produced significant changes in glucaric acid excretion (Table 2) (*65–71*). Intraindividual variation, which can be as high as 50%, may reflect daily variations in environmental exposures (*72*). Such variations would suggest that daily measures during vulnerable points in pregnancy would be required to assure accurate classification of exposure through the pathways that increase glucaric acid excretion. Laboratory methods for measuring urinary glucaric acid have been developed and standardized (*9*). However, standards for pregnant women do not yet exist.

Thioethers

Alkylating agents can be detoxified by reaction with glutathione or other sulfhydryl compounds. These conjugates frequently appear in urine as mercapturic acids or other thioether (*R-S-R*) products. Xenobiotics known to be detoxified through this sequence include aromatic hydrocarbons, arylamines, and many other chemical agents (*9*). Seutter-Berlage et al. (*73*) first proposed using urine thioethers as a screening tool for xenobiotic exposures. Since then, numerous studies have documented elevations in urinary excretions related to occupational, environmental, and behavioral (i.e., smoking) exposures (Table 3) (*73–104*).

Henderson et al. (*105*) and Van Doorn et al. (*76*) have critically reviewed the literature on the urinary thioether assay as an exposure screening tool. They note that positive results reflect true exposures, but negative results may not reflect lack of exposure. This false negativity occurs because thioethers measure short-term exposure and thus may miss past exposures that could have a future biological effect. This limitation, although perhaps not as great for exposures in pregnancy (because short-term exposures may be the most valid for measuring fetal exposure), suggests prospective urine collection at several points during pregnancy.

Porphyrins

Urinary porphyrin patterns are assessable through automated laboratory methods using high-pressure liquid chromatography (*106*). Brewster (*9*) has reviewed the usefulness of measuring total urine porphyrins to detect xenobiotic exposures to heavy metals, hormones, drugs, and halogenated aromatic hydrocarbons. These chemicals induce chronic disturbances in hepatic synthesis of porphyrins (Table 4) and thus lead to excess porphyrin excretion and skin symptoms in the final stage (*107–124*). Presumably, all xenobiotics that produce chronic changes would also show the urinary pattern at early stages prior to overt toxicity, but this assumption has not been tested in all circumstances.

Strengths and Limitations of Proposed Screening Battery

These four tests discussed previously generally meet the criteria for useful exposure screens. For certain xenobiotic agents, they accurately differentiate exposure levels, as demonstrated in occupational and environmental epidemiologic studies. As urinary screens, they are noninvasive and applicable on a large scale with current laboratory techniques. For short-term exposure, glucaric acid, thioethers, and mutagenicity tests are useful. Porphyrin patterns may measure cumulative effects as well as current exposure levels.

The potential for this battery to identify groups of pregnant women at risk of environmental insults to their fetuses can be illustrated by cigarette smoking. Glucaric acid may (*23*) or may not (*125*) be elevated by cigarette smoke. However, thioethers are elevated by cigarette smoke (*73,80,90,91*). Also, mutagenicity tests are very sensitive to cigarette smoking (*10*). Although cotinine is a specific marker for cigarette smoking (and thus would be the biomarker of choice if cigarette smoking is the specific exposure of interest), the fact that this battery is responsive to a known fetotoxic agent lends credence to its potential value for detecting other, as yet unknown, fetotoxins. However, as we have previously commented (*8*), to be effective screening

Table 3. Reported alterations in urinary excretion of thioethers by xenobiotics.

Effect[a]	Study	Reference
↑ Group mean	Chemical workers (possible exposure: acrylonitrile and biphenyl)	Seutter-Berlage et al. (*73*)
↑ Group mean	Rubber and tire workers	Salvolainen and Vainio (*74*)
↑ Group mean	Pesticide packaging workers	Seutter-Berlage et al. (*75*)
↑ Across shift	Operators of chemical waste incinerators	Van Doorn et al. (*76*)
↑ Across shift	Spinners in viscose-rayon	Van Doorn et al. (*77*)
↑ From pre-employment	Rubber industry (carbon disulfide) women	Kilpikari and Salvoainen (*78*)
↑ Group mean	Nurses handling cytotoxic drugs (cyclophosphamide, vincristin, cytoxan)	Jagun et al. (*79*)
↑ Group mean	Explosives manufacturers (trinitrotoluene)	Ahlberg et al. (*80*)
↑ Group mean	Oncology nurses (cyclophosphamide, adriamycin)	Que Hee et al. (*81*)
↑ Group mean and across shift	Petroleum retailers and garage mechanics	Stock et al. (*82*)
↑ Across shift	Dry cleaning workers	Lafuente and Mallol (*83*)
↑ Group mean	Urban school children in smoke-polluted areas (polcyclic aromatic hydrocarbons)	Lafuente and Mallol (*84*)
↑ Group mean	Oncology nurses handling cytotoxic drugs	Bayhan et al. (*85*)
↑ Across work week	Road and asphalt plant workers (asphalt)	Lafuente and Mallol (*86*)
↑ Across work shift	Chemical plant workers (3,3′ -dichlorobenzidine)	Triebig et al. (*87*)
↑ Across work shift	Hospital employees handling cytotoxic drugs	Triebig et al. (*87*)
↑ Post therapy	Cancer patients receiving cytotoxic drugs	Triebig et al. (*87*)
↑ Across work shift	Chemical plant workers (ethylene oxide, epichlorohydrine, formaldehyde, organic solvents including toluene)	Hagmar et al. (*88*)
↑ Group mean	Workers producing polyurethane foams (isocyanates and tertiary amines)	Holmen et al. (*89*)
↑ Group mean	Cigarette smokers	Seutter-Berlage et al. (*73*) Ahlborg et al. (*80*) Van Doorn et al. (*90*) Buffoni et al. (*91*) Heinonen et al. (*92*) Lafuente and Mallol (*93*)
→ Group mean	Chemical manufacturing workers (methylchloride)	Van Doorn et al. (*94*)
→ Group mean	Pharmaceutical manufacturing workers	Ahlborg et al. (*80*)
→ Group mean	Workers at waste water treatment plant (chlorinated cyclodiene pesticides and flame retardants)	Que Hee et al. (*95*)
→ Group mean	Crews of roll-on, roll-off ships and car ferries; bus garage staff (particulates, benzene, formaldehyde, NO_2, benzo(*a*)pyrene)	Ulfvarson et al. (*96*)
→ Group mean	Road asphalt plant workers (asphalt)	Burgaz et al. (*97*)
→ Across shift	Roadmen (PAHs)	Triebig et al. (*87*)
→ Across shift	Asphalt production workers (PAHs)	Triebog et al. (*87*)
→ Across shift	Petrochemical plant workers (benzene)	Triebig et al. (*87*)
→ Across shift	Coke plant workers (PAHs)	Triebig et al. (*87*)
↑	Toluene and xylenes in rats	Van Doorn et al. (*98*)
↑	1,3-Dibromopropane in rats	Onkenhout et al. (*99*)
↑	2-cl-and 3-cl-benzylidene malonitrile and benzaldehyde metabolites (chloro BMNs) in rats	Reitveld et al. (*100*)
↑	Ethylene dichloride (1,2-dichloroethane) in rats	Igwe et al. (*101*)
↑	*trans*- and *cis*-epoxy cinnamates in rats	Rietveld et al. (*102*)
→	Trichloroethylene in rats	Rouisse and Chakrabarti (*103*)
↑	Benzene, naphthalene, anthracene, phenanthrene, benzanthracene, styrene, aniline, 2-naphthylamine, acetophenetidine, halobenzenes, 1-chloronaphthalene, halonitrobenzenes, benzyl chloride, phenethyl bromide, 1-menaphthyl chloride, benzyl acetate, 1-menaphthyl acetate, 3,5-di-tert-butyl-4-hydroxytoluene, iodomethane, bromoethane, allyl chloride, 1-nitropropane, cyclopentene, bromocyclohexane, maleic acid, isolvaleric acid, ethyl methanesulfonate, urethane, benzothiazole-2-sulfonamide, *bis*-β-chloroethyl sulfide, ethacrynic acid arocoline, allyl acetate in various animals	Boyland (*104*)

[a](→) Unchanged.

Table 4. Reported alterations in porphyrin excretion by xenobiotics

Effect[a]	Study	Referenced
Abnormal pattern	Polyvinylchloride workers (vinylchloride)	Lange et al. (*107*)
Abnormal pattern	Michigan farm families exposed to polybrominated biphenyls (PBBs), 2 years prior	Strik et al. (*108*)
Abnormal pattern	Seveso explosion 2 years prior, dioxin exposure	Strik et al. (*109*)
Abnormal pattern	Occupational exposure to 2,4,5-T	Strik et al. (*110*)
↑ All porphyrins	Transformer/condenser production workers exposed to polychlorinated biphenyls (PCBs)	Colombi et al. (*111*)
↑ Uro	Turkish residents ingesting hexachlorobenzene in wheat, 20–30 years prior	Cripps et al. (*112*)
↑ Mean uro	Residents of Times Beach, Missouri, in dioxin exposure risk group	Hoffman et al. (*113*)
↑ Uro	Persons exposed to smoke from a PCB transformer fire	Octerloh et al. (*114*)
↑ Copro III	Lead-exposed workers	Tohiba and Tomokuni (*115*)
↑ Copro	Alcohol ingestion	Doss (*116*)
↑ Total porphyrins	Children exposed transplacentally to polyhalogenated aromatic hydrocarbons (PCBs, PCDFs, PC quaterphenyls)	Gladen et al. (*117*)
Normal pattern	PCBs exposure via contaminated rice oils, 10 years prior (Yusho)	Strik et al. (*118*)
Normal pattern	Possible occupational exposure to hexachlorocyclopentadiene, allyl chloride, epichlorohydrin, endrin	Nagelsmit et al. (*119*)
↑ Uro and copro	Hexachlorobenzene in rats	Kondo and Shimizu (*120*)
↑ Uro	PCBs, PBBs (20 different congeners) at least 2 Cl or 2 Br at lateral adjacent positions each phenyl ring; negative in <2 or >3 halogens each ring, in CEL cells[b]	Sassa et al. (*121*)
↑ Uro	3,4,5,3′,4′,5′ - Hexachlorobiphenyl, lindane, parathion, nifedipine, verapimil in CEL cells	Sinclair et al. (*122*)
↑ Uro and Hepta	3,4,3′,4′ - Tetrachlorobiphenyl in CEL cells	Sinclair et al. (*122*)
↑ Uro	Chlorobenzenes (4 chlorines) in CEL cells	Sinclair et al. (*123*)
↑ Uro	Aroclor 1254, oxidized 3,5-diethoxycarbonyl collidine, sodium phenobarbitone in CEL cells and embryos *in ovo*	Ferioli et al. (*124*)
↑ Copro III	Lead in rats	Tohiba and Tomokumi (*115*)

[a](↑) Increased concentration. Uro, uroporphyrin; hepta, heptacarboxygloporphyrin; copro, coproporphyrin; Copro III, coproporphyrin III.
[b]CEL, chick embryo liver cells in culture.

tools for adverse reproductive health exposures, several steps have yet to be taken.

First, tests must be standardized for pregnant women. Although there is little evidence to suggest that pregnancy itself can alter these test outcomes, it is important to establish standard levels for pregnant women with normal pregnancy outcomes. Second, tests should be administered to women with known exposures, such as maternal smoking, so that patterns of alterations can be correlated with reported exposures. Third, the tests must be associated with adverse pregnancy outcomes, such as reduced birthweight or gestational length. This last element in the validation research is particularly important since maternal exposure rather than fetal exposure is being measured. The extent to which xenobiotic chemicals cross the placental barrier may vary greatly, depending on the type of exposures, timing in pregnancy, and maternal detoxification capability. If the battery of screening tests proves useful, further field investigations would be warranted to determine the tests' ability to measure environmental exposures that adversely affect fetal development.

In reproductive epidemiology, we may be at a unique point for implementing this validation process. Because a number of studies of early pregnancy loss are collecting serial urines during pregnancy, the moment may be opportune to begin examining these urines for metabolic alterations, as tests of the potential usefulness of these nonspecific biomarkers to predict adverse pregnancy outcomes. Progress is being made in learning about these tests' response to specific environmental chemicals, but more research needs to focus on the quantitative relationship of these agents to body burdens. It would be helpful if this battery of tests were routinely applied to pregnant women in known exposure situations. Also, if pregnant women with abnormal tests (with and without adverse outcomes) were investigated further, much could be learned about the metabolic functions that are affected and the specific chemicals that are creating the effect.

Conclusion

Without better exposure measures, epidemiologic studies of reproduction will probably fail to identify xenobiotic fetotoxic agents in the environment. However, with an adequate battery of nonspecific exposure biomarkers, prospective studies of environmental effects on pregnancy outcomes might be possible. A proposed battery of nonspecific biomarkers should be tested to determine their usefulness for predicting adverse pregnancy outcomes.

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