Chapter 41
Do environmental contaminants including endocrine disruptors impair human sperm production and fertility?

Identification and regulation of man-made chemicals that may have adverse reproductive effects in men

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What is the evidence that environmental contaminants may alter sperm production and function in humans?

Back in the 1970s, a time when environmental risks to reproduction focused largely on females, a group of women whose husbands worked in a plant manufacturing the fungicide DBCP (dibromochloropropane) discovered that they were all having trouble getting pregnant. Subsequent occupational health investigations found an association between the men's job-related exposure to DBCP and low sperm counts or even azoospermia. Furthermore, sperm production improved in most men when the exposure ceased. This rather serendipitous finding raised awareness that chemical exposures may be harmful for men as well as pregnant women.

Subsequent studies in small cohorts of men exposed to relatively high levels of chemicals in occupational settings, such as agricultural or industry workers, or via accidental chemical releases, raised the level of interest in male reproductive hazards in the workplace, motivating the National Institute of Occupational Safety and Health to conduct and fund male-specific research. Since then, occupational health studies have provided strong evidence that men exposed to a variety of workplace chemicals, including certain industrial solvents, pesticides, plasticizers, surfactants (e.g., stain repellants), flame retardants, and metals (notably lead and cadmium) can be at increased risk of lower sperm counts and/or semen quality.

In contrast, it has been more difficult to study and find convincing evidence of adverse effects in men from the general population where exposures to related environmental contaminants in air, water and/or food are typically at low levels relative to occupational exposures and are often accompanied by the simultaneous presence of other contaminants in the environment. The challenge of attributing abnormal semen quality to any specific environmental exposure is confounded by many other factors that can affect semen quality adversely. These include the man's age, body mass index (BMI), stress levels, and/or habits such as smoking or drinking alcohol. Cohort and population-based studies designed to detect associations between an environmental exposure of concern and a male reproductive outcome must also consider the timing and duration of the exposure, and the nature of the contaminant. For example, it may be easier to detect the effects of a chemical that bioaccumulates over time, such as the pesticide DDT, than a chemical the is rapidly metabolized such as Bisphenol A (BPA). Thus, evidence from studies in human populations, while highly relevant, is often inconclusive, making it of limited use for supporting regulatory actions.

Then how do regulatory agencies evaluate the safety of chemicals and other environmental contaminants with respect to potential male-specific reproductive effects?

The US Environmental Protection Agency (EPA), established in 1970, is charged with protecting human health and the environment by enforcing environmental laws enacted by Congress. Pioneering environmental laws passed in the 1970s and updated since then, include the Toxic Substances Control Act, the Federal Insecticide, Fungicide and Rodenticide Act, the Safe Drinking Water Act, the Food Quality and Protection Act, and the Clean Air Act. These laws, and similar laws in other countries, require industry to test chemicals for toxicity to ensure their safety. It is then largely up to the States and other local governments to enforce these rules. Although some chemicals were initially grand-fathered-in for use (meaning they did not need to be tested for toxicity), recent updates in the US laws now require that all new and existing chemicals must pass tests for safety before they are registered and approved for sale, distribution, and use, or approved for new uses (https://www.epa.gov/assessing-and-

managing-chemicals-under-tsca/frank-r-lautenberg-chemical-safety-21st-century-act).

Early protocols designed to test for adverse reproductive effects (male and female combined) involved exposing groups of experiment animals (rats or mice) continuously to several dose levels of the chemical across 2 or more generations. In these so-called Multigenerational Test designs, young adult male and female animals are exposed for at least one spermatogenic cycle and then mated. The production of litters is measured, including the number and normalcy of the pups. The pups are then dosed through puberty and mated again to assess fertility and litter size and quality. This traditional approach was improved in 1998 to include more specific measures of male reproductive function such as diagnostic histology of the testis, sperm concentrations in the testis and epididymis, sperm motility and sperm morphology, the latter enabling more direct comparison with human studies based on semen quality (http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health Effects_Test_Guidelines/Series/870-3800.pdf). These guidelines have now been harmonized across federal agencies in the US and internationally through collaborations with the Organization for Economic Cooperation and Development (OECD) whose members include Canada, Mexico, most of Europe, Japan, Korea, Israel and Australia.

Even so, it is now widely accepted that the multigenerational test approach has a number of serious limitations:

- These protocols are expensive to conduct as well as time-consuming (months at least) and are therefore not feasible for screening the large number of chemicals in production or use today and the thousands of new chemicals requiring safety evaluations each year;
- They require the use of many animals resulting in ethical concerns;
- They are limited in sensitivity because they typically include only 3 dosages (high with general toxicity, medium, and low with little or no effect) making them ill-suited for defining dose response at environmentally relevant concentrations;
- They provide little if any mechanistic information that could inform risk assessment and/or be extrapolated to related chemicals;
- Because they expose animals for an entire reproductive cycle, they may detect only apical endpoints without providing

information about the initial cellular targets or biological mechanisms that might inform assessment of other similar chemicals;
- Importantly, they are not designed to detect so-called "endocrine disruptor chemicals" or EDCs which may act at very low exposure levels to perturb reproduction via hormone-mediated modes of action. Exposures to EDCs during embryo-fetal development and early postnatal development can alter reproductive tract development and thereby increase the risk of infertility later in life (after puberty) or increase risks for certain reproductive tract cancers.

Recognizing these limitations, and the potential benefits of using new information from genomics, epigenomics, bioinformatics, metabolomics and systems biology to advance the field of toxicology, the research and risk assessment offices of the EPA, Canada and other countries and in partnership with OECD launched a revolution in toxicity testing in the early 2000s called "Toxicity Testing in the 21st Century." This effort has generated and continues to develop and evaluate New Alternative Methodologies (NAMs) for toxicity screening, chemical prioritization, testing, and risk assessment to meet the need for better and more efficient chemical evaluation, including tests that are specific to male reproductive health.

What are NAMs and how can they be targeted to detect and characterize risks of chemicals specific to male reproduction?

NAMs are an evolving collection of innovative in silico, molecular, in vitro, and short-term in vivo tests and computational approaches designed to enable more rapid identification of potential health risks of chemicals and/or to prioritize those with the greatest potential for harm for further testing. With NAMs risk assessors and toxicologists are provided with new ways to evaluate the health effects of chemicals that are more efficient and more mechanistically driven. Rather than first testing chemicals in animals, chemicals are prioritized and grouped based on what is already known about their chemical structure or biological activity and then tested using biochemical, molecular or in vitro tests that screen them for their ability to act as an initiator of, or trigger for, a biological change or disruption. Changes in cells can then be linked to changes in whole

organisms that are indicative of reproductive dysfunction such as decreased sperm production by the testis. Adverse Outcome Pathways are constructed over time, and form the basis for further, more organ- or system-specific testing.

The EPA launched its EDC screening and testing program to develop and validate NAMs specific to identifying chemicals with endocrine activity (https://www.epa.gov/endo), specifically activity that disrupts estrogen, androgen, or thyroid action. This effort is being extended globally in partnership with OECD. Of relevance to male reproductive toxicity, chemicals with EDC activity can now be identified using in vitro androgen receptor binding and transactivation assays, and/or cell lines that synthesize testosterone in vitro. With such assays, hundreds if not thousands of chemicals can be screened in short order to prioritize positives for further testing in animals.

Furthermore, this program validated short term animal tests that can now be used to confirm results of in vitro screening. For male reproductive toxicity, the Hershberger Assay, for example, uses immature (prepubescent) male rats with undeveloped prostate glands.Control rats are given exogenous testosterone that causes the prostate gland to grow; increased prostate weight is indicative of a positive response within only a few days. Test animals are given suspected androgenic chemicals and their prostate weights compared with the testosterone-stimulated control weights. Conversely, a chemical with anti-androgen activity can be identified by its ability to block this response when administered together with testosterone. Thus, this test is useful for detecting chemicals with either androgenic or anti-androgenic activity. Finally, chemicals positive in in vitro screening and/or the short-term in vivo tests can then be moved directly into a risk assessment to support regulatory decision making. Thus, these new approaches are not only more rapid than multigenerational tests, but they use fewer animals and provide mechanistic information for risk assessment.

How might chemicals impact male fertility via endocrine disruption?

The reproductive system is exquisitely sensitive to disruption by EDCs during critical developmental windows. After puberty, the male reproductive system continues to depend on optimal levels of androgens and estrogens, and can therefore be disrupted by exposures to environmental anti-androgens and/or estrogens,

albeit usually requiring higher levels than those known to act in utero. For example, the later stages of spermiogenesis depend on androgen, and there is increasing evidence for a role for estrogen in sperm transport and function. At environmental levels of these contaminants, decrements in sperm production, transport, or function may be subtle and difficult to detect. However, even subtle effects may exacerbate reproductive problems in men if they are already subfertile for other reasons.

Toxicology studies using both traditional and NAM-based testing have shown that the fungicide vinclozolin (and its metabolites) inhibits androgen action by binding to the androgen receptor. Some phthalates interfere with steroidogenic pathways resulting in reduced testosterone levels. If women who are pregnant with male fetuses are exposed during critical windows of reproductive system development, their babies may be at increased risk of being born with hypospadias (incomplete closure of the penile urethra) or testicular maldescent (both repairable with surgery) (Chapter 44). In adults, higher exposures to EDCs may also inhibit androgen biosynthesis or receptor binding and thereby dampen testosterone-dependent processes including sperm maturation with potential effects on fertility. EDCs may diminish other testosterone-dependent functions including erectile function, cardiovascular health and metabolic health.

Exposure of men to estrogenic EDCs such as bisphenol A (and structurally related "BPA replacements") has been associated with abnormal semen parameters, reduced libido and erectile/ejaculatory difficulties as well as increased risk of prostate cancer. A variety of EDCs, including certain persistent pesticides (DDT), PCBs, dioxins, flame retardants, perfluorinated compounds, and organotins) and non-persistent EDCs such as BPA and other phenols, phthalates (used as plasticizers and in some personal care products), parabens (used as preservatives in some personal care products), pesticides such as organophosphates, pyrethroids and carbamates) and solvents (e.g., glycol ethers) have been reported to have negative effects in men or test species, albeit at higher exposures than during prenatal development, and have been banned or are under regulatory consideration based on their male reproductive effects.

Can chemicals have direct (non-endocrine mediated) effects on spermatogenic cells and are such effects reversible?

Environmental contaminants may also act directly on spermatogenic cells in the testis, or maturing sperm in the epididymis, acting via a variety of cellular-molecular mechanisms. For example, fungicides that act as spindle poisons such as carbendazim, may arrest spermatogenesis at meiotic stages resulting in low sperm concentrations. NAMs that evaluate cell division or microtubule function in non-reproductive cell assays would therefore be expected to detect potential testicular toxicants which could then be tested in short term in vivo tests for testis-specific effects.

Reactive chemicals that induce oxidative stress can also damage sperm DNA. For example, components of air pollution, polyaromatic hydrocarbons, can react with DNA in late-stage spermatids or epididymal sperm forming DNA-adducts. Exposure of men to intermittently high air pollution has been associated with DNA damage in mature sperm chromatin. Such damage cannot be repaired in the male tract and may not be repairable in fertilized eggs. In such cases, embryos may fail to develop resulting in early pregnancy loss and infertility. The good news is that the damaged sperm will soon be replaced by new, undamaged sperm as spermatogenesis proceeds, provided the exposure is of short duration. Using new tiered testing schemes, in vitro tests using non-reproductive cells that detect DNA adducts, and/or reactive oxygen species can now be used to screen for potential spermatotoxicants with similar activity.

A few male reproductive toxicants are known to act only or specifically on sperm maturing in the epididymis. Alpha-chlorohydrin, derived from certain industrial processes, is one such chemical. It specifically inhibits sperm metabolism with consequent inhibition of sperm motility and resultant infertility. Such effects are typically reversible once the exposure ceases.

Other toxicants can have multiple effects on spermatogenic cells, mature spermatids or sperm in the epididymis depending upon the dose and duration of exposure. Acrylamide monomer, for example, has male reproductive toxicity in mice and rats when tested using an acute, 5-day exposure protocol. At relatively high doses, its metabolites can arrest spermatogenesis resulting in temporary infertility due to low sperm counts and with recovery after the chemical has been metabolized. At lower concentrations

that do not arrest spermatogenesis, it can alkylate sperm chromatin which may cause early embryo loss and consequently reduced litter size, effects that are reversible after the exposure is removed. Interestingly acrylamide is also a neurotoxicant and acute exposure can interfere with the motor control of breeding in rats. Based on these studies, acrylamide has been regulated to prevent hazardous exposures in the workplace or releases into the environment.

Conclusion

With increased awareness about the potential of chemicals to negatively influence many biological processes, including spermatogenesis and fertility, international research and regulatory bodies have launched a revolution in toxicity screening and testing. This revolution is developing NAMs that are more sensitive, diagnostic, and efficient while at the same time less expensive and use fewer or no animals. By pairing NAMs with what we learn about the mechanisms through which chemical exposures might affect male reproductive function, future testing can better ensure that dangerous chemicals are removed from the environment or never enter commerce to begin with. The hope is that international cooperation will continue to support new research and provide the political will needed to insure environmental health protection of male reproductive health.

Suggested reading

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