

## What are the effects of environmental toxicants and/or endocrine disruptors on sperm function?

*Chemical exposure effects on the male, reactive oxygen species and spermatozoa*

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### What is the evidence that environmental contaminants may impact sperm function in humans?

Our appreciation for the potential impact of chemicals and other environmental contaminants on male reproductive function (spermatogenesis and fertility) was awakened in the 1970s when 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 epidemiological investigations showed an association between the men's 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 infertility was not necessarily a female problem and motivated regulatory agencies like the US EPA (Environmental Protection Agency), USDA (United States Department of Agriculture) and FDA (Food and Drug Administration) to improve their testing procedures and protocols in order to detect potential male reproductive toxicants. It also motivated NIOSH (National Institute of Occupational Safety and Health) and others to conduct occupational health studies for a variety of chemicals. We now know that in addition to certain pesticides, many environmental contaminants including certain industrial solvents, plasticizers and metals (notably lead and cadmium) have the potential to disrupt spermatogenesis and or specifically alter sperm function, given a sufficiently high exposure.

In general, evidence for adverse reproductive effects in humans has been found only in small cohorts of men exposed to relatively high levels of chemicals in occupational settings or from accidental chemical release. The extent to which the low levels of compounds or combinations of compounds that we encounter in ambient day-to-day environments may impact male reproduction is largely unknown and far more difficult to determine.

## How do regulatory agencies test chemicals for spermatotoxicity and predict male reproductive effects?

In 1998, the US EPA updated its testing protocols required for the ([http://www.epa.gov/opptsfrs/publications/OPPTS\\_Harmonized/870\\_Health\\_Effects\\_Test\\_Guidelines/Series/870-3800.pdf](http://www.epa.gov/opptsfrs/publications/OPPTS_Harmonized/870_Health_Effects_Test_Guidelines/Series/870-3800.pdf)) registration of pesticides. The current guidelines, that have been harmonized across federal agencies in the US and internationally, call for multigenerational testing in animal test species (primarily rats) where measures of sperm production and function (sperm motility and morphology) are obtained in parental adult animals and in their offspring. The protocol also evaluates reproductive tract development after *in utero* or early life exposures. Because males are dosed for the entire duration of spermatogenesis before they are evaluated and bred, adverse effects on any spermatogenic or somatic cell target should be detectable in the form of reduced testis or epididymal weights, altered testis histology (one of the most sensitive indicators of effects), reduced epididymal sperm counts, altered sperm motility or morphology and/or reduced fertility. When effects are seen, additional specific tests for sperm function such as *in vitro* fertilization (IVF) and evaluation of sperm chromatin structure, DNA damage, and chromosome numbers/structure can be used to further characterize the effect (Chapter 13).

Although typically not required for non-pesticidal industrial chemicals, these standard and specialized tests are being applied on a voluntary basis to evaluate chemical safety for compounds produced and used in high volume and those predicted to have reproductive effects based on their similarity to known toxicants (e.g. by structure-activity analysis) or based on *in vitro* screening for endocrine activity, oxidative stress, mutagenicity or cytotoxicity.

Regulatory agencies consider all available information from such tests when they perform a risk assessment, the complex process that integrates dose-response data from the test species, the chemical's mode of action (if known), the relevance of the animal data to humans, and any available human epidemiological data with the goal of predicting risk to humans. In addition to formal risk assessments conducted by regulatory agencies, the National Toxicology Program conducts reviews of high priority reproductive toxicants through its Center for Environmental Risks to Human Reproduction (CERHR) and posts reports on the internet (<http://cerhr.niehs.nih.gov/>). CERHR selects chemicals based on both their occurrence (production volume and likely human exposure) and mode of action (likely reproductive

toxicant), thereby evaluating compounds of high public and regulatory concern such as acrylamide, bromopropane, phthalates and bisphenol A.

### **Biologically plausible modes of spermatotoxicity: Oxidative stress**

Sperm are unique in their metabolism and actually use oxidative stress to advantage. Reactive oxygen species (ROS) generated by metabolically active sperm play a role in the normal signaling that leads to capacitation or the capability of fertilizing eggs (Chapter 15). However, excess ROS can also cause membrane damage (lipid peroxidation) and sperm DNA damage, with undesirable consequences on fertilizing ability and genetic integrity of the ensuing embryo, respectively. Therefore, environmental contaminants that generate oxidative stress or alter REDOX status (including DBCP, acrylamide and bromopropane) are biologically plausible candidate spermatotoxicants. An understanding of oxidative stress in sperm is also directly applicable to assisted reproductive technologies. Because sperm can undergo oxidative damage during *in vitro* culture, media components and IVF procedures are being optimized to minimize such effects.

Few known male reproductive toxicants act only or specifically on sperm maturing in the epididymis. Alpha-chlorohydrin, a sperm metabolic poison that inhibits sperm motility, is one such chemical. However, some toxicants can have direct effects on sperm motility and/or fertilizing ability and impair earlier stages of spermatogenesis. For example, chronic exposure to acrylamide can damage spermatogonia and spermatocytes and indirectly impair fertility by arresting spermatogenesis. At levels that do not arrest spermatogenesis, the reactive metabolite of acrylamide can also alkylate sperm nuclear protamine during the final stages of spermiogenesis. As a result, the sperm may be able to fertilize eggs, but the resulting embryos die due to acrylamide-induced damage in the sperm pronucleus. The good news is that effects of chemicals that react directly with maturing sperm are as transient as the exposure. Once the exposure has ended and the affected sperm have been released from the body, the risk disappears.

Cigarette smoke and air pollution resulting from the combustion of coal and gasoline contain reactive chemicals that can cause oxidative stress and inflammatory changes in the respiratory and cardiovascular systems. Both cigarette consumption and exposure to high levels of air pollution have also been associated with altered semen quality and DNA damage in sperm. Because such sources of oxidative stress can be difficult to avoid, antioxidant dietary supplements may be of therapeutic or protective value. However, convincing benefits of this strategy have been hard to measure in human studies.

### **Biologically plausible modes of spermatotoxicity: Endocrine disruptors**

Certain chemicals may impair sperm function by interfering with androgen action. For example, toxicology studies in rats show that the fungicide vinclozolin (and its metabolites) inhibits androgen action by binding to the androgen receptor, and some phthalates interfere with steroidogenesis resulting in reduced testosterone synthesis. Some chemicals such as bisphenol A can act as estrogens, while others are promiscuous and bind multiple receptors. Because hormones themselves are active at very low levels, these so-called “endocrine disruptors” have the potential to impact human reproductive processes, even when present at the low levels typically encountered in the environment. Furthermore, the developing male reproductive system is particularly sensitive to altered testosterone signaling. Toxicology studies in rats, where specific windows of exposure and sensitivity can be precisely defined, have shown that blocking androgen action during reproductive tract development can result in hypospadias and testicular maldescent in male offspring. Since the later stages of spermiogenesis depend on androgen, and there is increasing evidence for a role for estrogen in sperm function, endocrine disruptors may also impact sperm function in adults. At environmental levels of these contaminants, such effects are likely to be subtle and difficult to detect. However, even subtle effects may have negative impacts on subfertile men who are thought to be more susceptible to environmental stressors.

Based on growing evidence about the occurrence and potentially adverse effects of environmental endocrine disruptors, regulatory agencies have been directed by Congress to develop an effective screening and testing program for contaminants with endocrine disruption potential (<http://www.epa.gov/endo/>). These screening tests and other high throughput test systems currently under development will make it more feasible to test the large numbers of new chemicals introduced into our environment by commerce and thereby protect our reproductive health.

### **How can clinicians advise their infertility patients and couples attempting pregnancy?**

Clearly there is a need for patient-oriented information about the linkage between environmental contaminants and risks to male reproductive function. The Collaborative on Health and Environment makes such information readily available on the web ([http://www.healthandenvironment.org/working\\_groups/fertility](http://www.healthandenvironment.org/working_groups/fertility)). At present, physicians can at least include a

short history on occupational and environmental exposures and advise their patients to avoid exposures to known toxicants such as cigarette smoke, solvents and pesticides.

### **Suggested reading**

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