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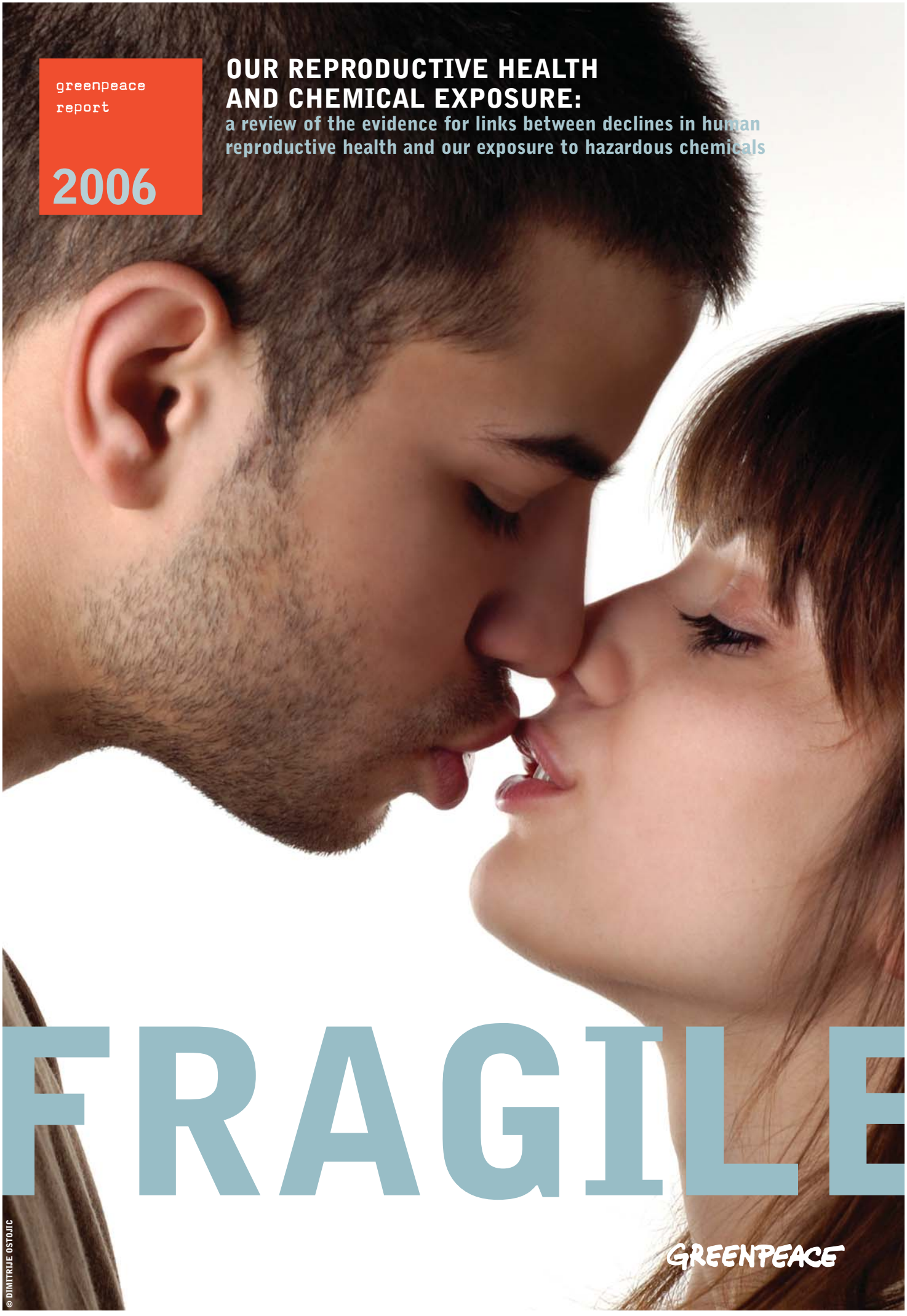
OUR REPRODUCTIVE HEALTH AND CHEMICAL EXPOSURE:

a review of the evidence for links between declines in human reproductive health and our exposure to hazardous chemicals



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published by Greenpeace International
date April 2006
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acknowledgements The authors would like to thank Fawaz al Bitar, Helen Perivier and Nadia Haiama for their review and comments, Madeleine Cobbing for her final edits and Helen Perivier and Martin Hojsik for coordination of the production of this report.
design & layout Tania Dunster, **one**hemisphere, Sweden

This report draws upon and updates information contained in two previous reports published by Greenpeace UK in 2003:

Dorey, C.N. (2003) Chemical Legacy – Contamination of the Child, Greenpeace UK, October 2003: 54 pp
http://www.greenpeace.org/international/press/reports/chemical-legacy-contaminatio

Greenpeace UK (2003) Human impacts of man-made chemicals, Greenpeace UK, September 2003: 17 pp
http://www.greenpeace.org.uk/MultimediaFiles/Live/FullReport/5988.pdf

We are indebted to them both.

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OUR REPRODUCTIVE HEALTH AND CHEMICAL EXPOSURE:

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executive summary

There is a growing body of evidence which indicates a disturbing rise in the incidence of disorders of the human reproductive system. For example:

* sperm counts have declined dramatically over the past 50 years in many countries
* testicular cancer has increased significantly
* infertility may now affect 15-20% of couples in industrialised countries compared to 7-8% in the early 1960s
* girls are reaching puberty at an earlier age in some parts of the world
* the incidence of endometriosis in women is high in some countries
* the number of boys born compared to the number of girls has shown declines in several regions, with marked changes in some areas.

The exact causes of the increase in reproductive problems are presently unknown. However, in parallel with the increase there has been a rise in the manufacture and use of many chemicals. It has been estimated that every year around 100,000 different types of chemicals are produced and used around the world. The use of chemicals has led to the inevitable contamination of the environment and consequently, also to human exposure. Many chemicals have been found to contaminate human tissues and even the developing foetus in the womb is exposed to a multitude of chemicals which pollute the human body.

Although not proven beyond doubt, there is increasing evidence of a possible link between the synonymous rise of reproductive health problems and the rise of our exposure to many chemicals. The presence of many man-made chemicals at current environmental levels may already be negatively impacting the reproductive health of wildlife and humans. The grounds for such a hypothesis draw on a number of lines of evidence, including laboratory studies on effects of chemicals in animals, direct measurements of chemical exposure in humans (including presence of chemicals in body tissues) and the findings of correlations between level of exposure to chemicals and incidence of certain disorders.

Laboratory studies have shown beyond any doubt that certain chemicals are capable of causing reproductive disorders in animals. Of particular concern in this regard are persistent organic pollutants (POPs) and other pervasive hazardous chemicals which are known to be toxic to reproductive health and/or disrupt the hormone (endocrine) system. While some of these chemicals, especially the internationally-recognised POPs, have been banned or severely restricted, other reproductive toxicants and chemicals that disrupt the endocrine system (endocrine disruptors) remain in use by industry and, in many cases, may still be found as ingredients or additives in a variety of household products. Among the known chemicals of particular concern with regard to human reproductive health are those listed in table 1.

Many of the reproductive disorders which have been increasing in incidence are thought to originate in the developing stages of the foetus's life in the womb. The nursing young may also be vulnerable. It is therefore of great concern to know that some of the chemicals listed above are able to cross the placenta and also contaminate breast milk. For example, alkylphenols, brominated flame retardants, artificial musks, phthalates and bisphenol A have been found to contaminate blood taken from the umbilical cord. Furthermore, two studies of phthalates in human babies have found evidence that these chemicals are associated with hormone disruption although further studies are needed to confirm the findings.

Classically, toxicology has focused heavily on lethal effects on test animals of high doses administered over short periods of time. Invariably, however, sub-lethal effects (i.e. adverse effects other than death) of acute exposure, as well as both lethal and sub-lethal effects of longer-term (chronic) exposure, are found to occur at doses well below the so-called LD50 (the dose found to be lethal to 50% of the animals exposed in any one test).

While we may never be certain that there are chemical causes behind the increasing trends of reproductive health problems, the evidence to date cannot be ignored. The most responsible way forward is to take precautionary action on these chemicals of concern. We urgently need laws that protect us from continued exposure to such chemicals.

Presently, new legislation is being drafted in the European Union to regulate the manufacture and use of chemicals (Registration, Evaluation, Authorisation of CHemicals, or REACH). It is within the capacity of the EU institutions (the European Parliament and the Council of the European Union) to formulate regulations that are based on precaution and substitution, such that hazardous chemicals (including reproductive toxicants and endocrine disrupting chemicals) can

be phased out of use and substituted with safer alternatives. Ultimately, if such a route can be taken by the EU it is to be hoped that other governments around the world will follow suit and phase out hazardous chemicals.

In addition, decision makers should ensure that sufficient data about the properties of chemicals are provided by the producers and importers to establish the full range of hazards posed, and identify chemicals which are possible endocrine disruptors or otherwise toxic to reproduction.

TABLE 1: KNOWN CHEMICALS OF PARTICULAR CONCERN WITH REGARD TO HUMAN REPRODUCTIVE HEALTH

CHEMICAL GROUP	COMMON USES	REPRODUCTIVE HEALTH CONCERNS
alkylphenols and related chemicals	* formerly[1] in industrial and institutional cleaning sector (including domestic cleaning) * textile and leather processing * personal care products * pesticide production	* hormone mimicking activities * reduced male fertility, testicular size, sperm quality
phthalates	* plasticisers in PVC and special polymer applications * gelling agents * solvents and fixatives in cosmetics and other personal care products	* testicular toxicity * reduced anogenital distance, cleft phallus, hypospadias and undescended testes in immature males * reduced male and female fertility * foetal toxicity (possibly leading to death or malformations)
brominated flame retardants	* as flame retardants in industrial and electrical appliances, vehicles, lighting, wiring as well as textiles, furnishing and insulating materials such as polystyrene	* oestrogen mimicking * birth defects in rodents documented * impacts on nervous system and behavioural development
organotin compounds	* PVC UV stabilisers * Agrochemicals and biocides * Antifoulants * Catalysts	* inhibition of steroid hormone production * adverse impact on in utero development of foetus including abnormalities in genital development in male foetuses
bisphenol-A and its derivatives	* production of polycarbonate plastic used e.g. in baby bottles, CDs, motorcycle windshields, etc * production of epoxy resins used in e.g. coatings in food packaging	* oestrogenic activity * altered male reproductive organs * early puberty induction * reduced breast feeding
artificial musks	* fragrance mixtures for detergents, fabric, conditioners, cleaning agents, air fresheners and other household products * cosmetic products such as soaps, shampoos and perfumes	* oestrogenic activity * anti-oestrogenic activity

1. Many uses of nonylphenol and it compounds were recently prohibited through Europe-wide restrictions on marketing and use (26th Amendment of Directive 76/769). Many uses of octylphenol and its compounds are apparently now subject to voluntary phase-outs by industry in advance of completion of formal risk EU assessment. Beyond Europe, there are few restrictions and use of both remains widespread, including in industrial and household detergents.

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introduction

Growing burden of a chemical world

It has been estimated that, every year, around 100,000 different types of chemicals are produced for a wide range of uses around the world (EEA, 1999). Estimates for the European Union (EU) range from 30,000-70,000. Many of these chemicals, especially those used in high tonnages and in open applications (i.e. not in industrial closed-systems) eventually find their way into our environment. Other chemicals that have been banned from manufacture and use for many years still leak from old products or are long-lived and linger in the environment, while new chemicals are synthesised and brought to the market every month.

As a result, we are now being exposed to tens of thousands of chemicals which simply didn't exist on this planet until a few decades ago. Studies have shown that many such chemicals can be found in the body tissues of wildlife and humans. In some cases, exposure can even occur during the most sensitive life stage – the developing foetus in the womb. Yet when our grandparents were in their mother's wombs, they would not have been exposed to these novel chemicals.

Several recent studies, including that conducted by the Environmental Working Group in the USA and by WWF and Greenpeace in Europe, have reported the presence of a wide array of man-made chemicals in the blood of adults and children (WWF-UK 2003, 2004, WWF 2004a, b, Greenpeace Netherlands 2004, Greenpeace/WWF 2005). None of the adult volunteers were known to receive regular exposure to chemicals as part of their jobs and yet their blood was still contaminated, illustrating just how ubiquitous our daily exposure to chemicals really is.

The placenta generally does not act as barrier to chemicals which already pollute the mother's body. Therefore the developing foetus can be exposed to chemicals in the mother's blood. As well as being exposed to those chemicals which the mother is exposed to daily, the developing foetus is also exposed to chemicals that have been stored in her tissues, and are released during pregnancy. The amniotic fluid that bathes the developing baby has also been found to contain hazardous chemicals, as well as the blood supply in the umbilical cord (Greenpeace/WWF 2005). After birth, the nursing infant can be further exposed to chemicals from the mother's body by breast milk[2].

These studies examined the distribution of several key chemical groups because of concern for their known potential to cause adverse effects (including interference with the hormonal system). However many other synthetic chemicals are likely to have been present which simply were not tested for.

Reproductive health in decline

The increase in the incidence of certain conditions of the reproductive system (and other health effects) has paralleled a rise in the manufacture and use of chemicals. For example, over many parts of Europe, sperm counts have decreased dramatically over the past 50 years, testicular cancer has increased significantly and the ratio of male to female births appears to be shifting.

The parallel rise of such health phenomena could be a coincidence. The methods we have at our disposal to determine the causes of diseases like cancer, or intergenerational impacts of chemicals that interfere with hormones, are inevitably limited in their ability to give us clear answers. What is clear, however, is that many chemicals commonly found in the environment and in human bodies, have shown themselves in laboratory tests to be capable of causing the type of effects which may underlie the trends in reproductive human health that we are witnessing across the globe. We may never be certain that there are chemical causes behind these trends, but the evidence calls for serious attention.

2. It is widely acknowledged that breastfeeding confers substantial benefits on babies, in the form of vital nutrients and antibodies passed from the mother to baby, especially in the first few months of life. It also helps the bonding process between mother and child. Therefore, in spite of concerns regarding chemical contamination, the advice from scientists and health professionals is to continue breastfeeding. Rather than being a reason to stop breastfeeding, the current presence of chemical contaminants in breast milk illustrates the urgent need to tackle chemical pollution at source.

Chemical interference with reproductive development

Many chemicals are of concern due to their impacts on reproductive health. A total of more than 50 chemicals are officially classified in Europe as toxic to reproduction (labeled R60, impaired fertility, or R61, harm to the unborn child) under Directive 67/548 on classification and labelling. Tens of others are recognised as potentially toxic to reproduction (labeled R62 or R63). But this is undoubtedly only a fraction of those chemicals on the market with the ability to interfere with reproductive development in animals, including humans. Many more are already known or suspected of being able to interfere with the endocrine (hormone) system, the chemical signaling mechanism in all of us which is so vital in controlling growth, development and health. More still have simply never been tested for such effects.

Until relatively recently, concerns focused on a small number of well known man-made environmental pollutants, including chlorinated pesticides like DDT, dieldrin, chlordane and hexachlorobenzene and other, once widely used, chemicals such as the polychlorinated biphenyls (PCBs). As well as being widespread through the environment, including ecosystems far from their source of use and release, these chemicals have been known for decades to exhibit a wide range of toxic effects on wildlife and, in some cases, humans, including impairment of reproductive development (Allsopp *et al.* 1999). It is only more recently that details of the mechanisms underlying their toxicities has come to light, including the phenomenon of hormone or endocrine disruption.

Even then, research has focused heavily on one particular effect, namely the ability of these man-made chemicals to mimic the natural female steroid hormones known collectively as oestrogens. Effects on other parts of the endocrine system are poorly investigated. Moreover, when it comes to the host of other hazardous chemicals to which we are exposed every day, limits to scientific understanding are greater still.

International recognition of the dangers presented to the environment and human health of some of the most widespread and persistent chemicals, the so-called Persistent Organic Pollutants (or POPs) including PCBs and the chlorinated pesticides listed above, led ultimately to the preparation of the Stockholm Convention (2001). With few exceptions, this handful of chemicals (12 in total) are now banned or severely restricted in their use in most countries across the globe, although our exposure to them continues because they are so long-lived and because, in the case of the dioxins and PCBs, they are still produced as unintentional by-products of ongoing industrial and waste management processes.

While recognising that proposals are on the table to add a few other chemicals to the Stockholm POPs list, they will always represent only a small fraction of those chemicals capable of damaging reproductive development. Other hazardous chemicals with similar properties of environmental persistence (resistence to degradation), ability to bioaccumulate (build up in body tissues and through the food chain) and toxicity (including toxicity to the developing reproductive system) remain in widespread production and use today. Some are even used as additives in products widely available to consumers in Europe. See table 2.

introduction *continued*

TABLE 2: KNOWN CHEMICALS OF PARTICULAR CONCERN WITH REGARD TO HUMAN REPRODUCTIVE HEALTH

CHEMICAL GROUP	COMMON USES	REPRODUCTIVE HEALTH CONCERNS
alkylphenols and related chemicals (especially alkylphenol ethoxylates, or APEs)	* formerly[3] in industrial and institutional cleaning sector (including domestic cleaning) * textile and leather processing * personal care products * pesticide production	* hormone mimicking activities * reduced male fertility, testicular size, sperm quality
phthalates	* plasticisers in PVC and special polymer applications * gelling agents * solvents and fixatives in cosmetics and other personal care products	* testicular toxicity * reduced anogenital distance, cleft phallus, hypospadias and undescended testes in immature males * reduced male and female fertility * foetal toxicity (possibly leading to death or malformations)
brominated flame retardants (especially the polybrominated diphenyl ethers, or PBDEs, hexabromocyclododecane, or HBCD, and tetrabromobisphenol-A, or TBBPA)	* as flame retardants in industrial and electrical appliances, vehicles, lighting, wiring as well as textiles, furnishing and insulating materials such as polystyrene	* oestrogen mimicking * birth defects in rodents documented * impacts on nervous system and behavioural development
organotin compounds (including mono, di and tributyltin and triphenyltin)	* PVC UV stabilisers * Agrochemicals and biocides * Antifoulants * Catalysts	* inhibition of steroid hormone production * adverse impact on *in utero* development of foetus including abnormalities in genital development in male foetuses
bisphenol-A and its derivatives	* production of polycarbonate plastic used e.g. in baby bottles, CDs, motorcycle windshields, etc * production of epoxy resins used in e.g. coatings in food packaging	* oestrogenic activity * altered male reproductive organs * early puberty induction * reduced breast feeding
artificial musks (including nitromusks such as musk xylene and musk ketone, as well as polycyclic musks such as HHCB, or Galaxolide, and AHTN, or Tonalide)	* fragrance mixtures for detergents, fabric, conditioners, cleaning agents, air fresheners and other household products * cosmetic products such as soaps, shampoos and perfumes	* oestrogenic activity * anti-oestrogenic activity

3. Many uses of nonylphenol and it compounds were recently prohibited through Europe-wide restrictions on marketing and use (26th Amendment of Directive 76/769). Many uses of octylphenol and its compounds are apparently now subject to voluntary phase-outs by industry in advance of completion of formal risk EU assessment. Beyond Europe, there are few restrictions and use of both remains widespread, including in industrial and household detergents.

It has been known for some time from laboratory studies that these chemicals, among others, have an inherent ability to interfere with the endocrine (hormone) system in animals, including mammals. In some cases, adverse effects on reproductive health are well documented, especially impacts on development of reproductive organs in early life stages. Hormones control developmental processes in the foetus and infant at low parts per trillion (ppt or one part in every million million) levels. As we will see below, many environmental pollutants can be found within human body fluids or tissues at substantially higher levels than this, such that even chemicals showing 'weak' hormone-disrupting activity may be of significance.

Because of the complex nature of the endocrine (hormone) system in wildlife and humans and the fact that it is controlled by very low doses of natural hormones circulating in the body, the toxicology of endocrine disruptors has proven particularly difficult to predict, describe and quantify. Nevertheless, given the range of developmental and metabolic processes which are controlled by hormones, the significance of exposure to chemicals able to interfere with their natural signalling mechanisms cannot be overstated.

Of further concern is the fact that we know little about the health implications of exposure to mixtures of many chemicals. Generally, in laboratory studies, the effects of exposure to chemicals are tested individually on a chemical by chemical basis. There are few means to test the toxic impacts from exposure to chemical mixtures, especially when such chemical 'cocktails' run to tens or even hundreds of individual substances.

As noted in the Prague Declaration, a statement issued by a group of international experts and scientists representing many different disciplines meeting at the EU-funded CREDO Cluster Workshop on endocrine disruption in Prague in May 2005:

"Europeans are exposed to low levels of a large number of endocrine disrupters which can act in concert. Many of these chemicals, drugs or natural products are found in human tissues and in breast milk. Humans are exposed to these chemicals from very early on in their lives when the developing organism can be particularly sensitive"
(Prague Declaration 2005).

Protecting the next generation

We are currently at a pivotal moment in the history of chemical regulation. At the end of 2006 the European Parliament and governments will decide on a proposed legislation known as REACH (Registration, Evaluation, Authorisation of Chemicals) that is intended to significantly improve the way in which chemicals are regulated and used across Europe. REACH initially promised major changes in order to ensure a high level of protection for human health and the environment by compelling industry to provide safety data for the chemicals they produce and by replacing the most problematic 'substances of very high concern' (including those toxic to reproduction) with safer alternative chemicals or technologies.

However, as drafting has proceeded, the legislation has become progressively weaker and less ambitious such that the degree of protection that it is likely to provide is currently under serious question. Whether the EU will choose to protect the public from hormone disrupting chemicals is also now at stake. The issue of whether or not it is possible to establish safe levels of chemicals that are active at minute levels and that have potentially additive combined effects is at the core of the debate. These issues are addressed in a separate report (Santillo and Johnston 2006).

The present report explores the consequences of our failure to date to control exposure to chemicals which are toxic to reproduction and, therefore, the implications for the future if REACH does not provide the level of protection required. It reviews trends in reproductive health more closely, along with the evidence for contributions to those trends from exposure to hazardous chemicals in daily life, using examples relating to the chemicals listed above to illustrate the problem. Further detailed information on the reproductive toxicity of these chemicals, as discerned from laboratory experiments, is also presented, as well as the available evidence on their widespread presence as contaminants in human tissues.

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reproductive health trends and chemical exposure: making the link

Direct evidence of health impacts in humans due to exposure to chemicals of concern discussed in this report is, and no doubt will remain, difficult to demonstrate. This is because there are no control groups without any chemical exposure with which to compare those who have chemical exposure – in fact, we are all exposed to multiple chemicals at widely varying levels. Furthermore, some diseases and other health conditions can develop many years or even decades after the key period of chemical exposure; even though the damage may be done at a very young age, the health consequences may not be realised until adolescence or adult life, further complicating the task of investigating the link between chemical exposure and health effects.

Laboratory studies can be used to determine whether chemicals can affect hormones, by looking for indicators of endocrine disruption and/or reproductive impairment in whole organisms. Human studies are rare, and obviously cannot involve deliberate exposure to toxic chemicals and the measurement of effects. Studies of impacts on humans have therefore inevitably focused on identifying relationships (correlations) between concentrations of different chemicals in the body and incidence of reproductive diseases.

Exposure during early stages of life is of particular concern. Both unborn and newborn babies are thought to be more susceptible to chemical exposure because they process and eliminate chemicals from their body more slowly than in adults, and because these periods represent some of the most complex and sensitive in terms of body development. Hormones play many critical roles in controlling growth and development in early life, such that any interference could have serious and irreversible effects on child development with consequences that may be felt throughout their later lives.

This section presents in more detail the evidence for trends in reproductive health of humans and possible links to chemical exposure.

2.1 Male Reproductive Health

2.1.1 Trends in Male Reproductive Health and Endocrine Disruption

A number of worrying trends in male reproductive health have been identified in industrialised countries:

* A paper published in 1992 reported that sperm counts had decreased worldwide by 50% between 1940 and 1990 (Carlsen *et al.* 1992). Since then other studies have also reported declines in sperm count in several countries. Sperm counts have been reported to be decreasing at a rate of about 1% per year (Swan *et al.* 2000). On average, a typical western man produces only half the sperm his father or grandfather did (Carlsen *et al.* 1995, Swan *et al.* 1997, Swan *et al.* 2000). For example, a study in Denmark showed that 18-20 year olds born around 1980 had the lowest sperm counts ever recorded in normal Danish men (Andersen *et al.* 2000). Studies in France, Scotland and Denmark have also shown that sperm quality is worse in younger men (Carlsen *et al.* 1992, Auger *et al.* 1995, Irvine *et al.* 1996, Carlsen *et al.* 1999, Andersen *et al.* 2000, Skakkebaek *et al.* 2001). Low sperm counts now affects approximately 20% of young men in some European countries (Sharpe 2005). Infertility may now affect 15-20% of couples in industrialised countries, compared to 7-8% during the early 1960s (Saradha and Mathur 2006).
* Studies at three fertility advice centers in Germany (Hamburg, Leipzig and Magdeburg) also revealed significant declines over time in sperm quality (Licht 1998, Glockner *et al.* 1998, Thierfelder *et al.* 1999). Comprising several thousand subjects in each case, these studies remain among the biggest so far conducted anywhere in the world.
* The incidence of a birth defect of the penis, where opening of the urethra occurs not at the top of the penis but along the shaft or scrotum (hypospadias), doubled in the USA between 1970–1993 (Paulozzi *et al.*1997).
* Cryptorchidism, where testicles fail to descend into the scrotum before birth, occurs in 2 -5% of male babies in Western countries, and is increasing. Males born with this defect are also at a higher risk from testicular and breast cancer in later life (Paulozzi, 1999).

* The incidence of testicular cancer is rising in some parts of the world. It is the most common cancer in men aged 20–34 years (Huyghe *et al.* 2003). The incidence of testicular cancer in Caucasian men has been increasing progressively for the past 50 years or more (Sharpe 2005). In the former German Democratic Republic regions of Germany, incidence of testicular cancer increased four-fold in the 28 years from 1961 to 1989, an annual increase of 5%, with the greatest increases occurring within urban populations (Rosch *et al.* 1999).

It is thought that the diseases and abnormalities of the male reproductive systems listed above may be the symptoms of a single disorder called testicular dysgenesis syndrome. A failure of normal development of the fetal testis in the womb, this condition is likely to be caused by a disruption of sex hormones during development (Skakkebaek *et al.* 2001). Specifically, the syndrome is thought to involve hormonal dysfunction of the fetal testis, in particular a reduced production of testosterone. This results in "undermasculinization" of the male child. There is a hypothesis that chemicals that interfere with hormones during development, including working against the action of male steroid hormones (so-called anti-androgenic chemicals), could result in these adverse impacts on the male reproductive system. Since humans are exposed to chemicals which can interfere with hormonal action, it is possible that exposure in the womb to such endocrine disrupting chemicals may be a contributing factor to the rising incidence of these male reproductive health problems.

A group of international experts and scientists representing many different disciplines came together in Prague on 10 – 12 May 2005 for a workshop on chemicals that interfere with the endocrine system (the CREDO Cluster Workshop). The resulting declaration issued by scientists at the meeting, the so-called Prague Declaration, highlights the high level of concern regarding declining male reproductive health and the possible contribution to this from exposure to endocrine disrupting chemicals:

"There is serious concern about the high prevalence of reproductive disorders in European boys and young men and about the rise in cancers of reproductive organs, such as breast and testis. Lifestyle, diet and environmental contamination play a role in the observed regional differences of these disorders and their changes with time".

... and ... ,

"Hormone action is important in the origin or progression of the aforementioned disorders. Therefore it is plausible that exposure to endocrine disrupters may be involved, but there are inherent difficulties in establishing such causal links in humans".

(Prague Declaration 2005)

The vast majority of chemicals in use have never been tested for possible effects on the androgen (male steroid hormone) system, despite the fundamental role this system plays in controlling growth and development in wildlife and humans.

2.1.2 Chemical exposure in the very young: the case of phthalates

To date, very few studies have focused directly on possible impacts of endocrine disrupting chemicals on human male reproductive health. Two of the most recent and most prominent of such studies (Main *et al*. 2006, Swan *et al.* 2005) relate to exposure in the womb and shortly after birth to phthalates (phthalate esters). Taken together, these studies suggest that exposure to phthalates in the early stages of life is associated with hormone-disruption and impacts on male reproductive health.

The study by Main *et al.* (2006) investigated whether phthalates in breast milk had any influence on the levels of reproductive hormones in boys at the age of 3 months. 68 healthy boys were monitored alongside 62 boys with cryptorchidism, a condition in which the testicles fail to descend into the scrotum before birth. Although the study did not reveal any direct association between phthalate exposure and incidence of cryptorchidism, it was found that higher levels of the phthalate metabolites monomethyl phthalate (MMP), monoethyl phthalate (MEP), mono-n-butyl phthalate (MnBP) and monoisononyl phthalate (MiNP) in breast milk, and therefore higher exposure in the newborn boys, were associated with alterations in their levels of reproductive hormones. These results supported the hypothesis that the human testis may be vulnerable to phthalate exposure during development, even if cryptorchidism is not a predictable consequence. The authors suggested that further research was urgently needed to confirm the findings and stressed that, while no link was apparent between exposure and cryptorchidism, the study group may simply have been to small to detect such an effect.

Studies on laboratory animals have conclusively shown that exposure of pregnant rats to certain phthalates results in a collection of reproductive disorders in male offspring which are similar to testicular dysgenesis in men (see section 3.2). The study by Swan *et al.* (2005) investigated whether human exposure to phthalates in the womb is similarly associated with effects on male reproductive health in the exposed child.

This study monitored the concentrations of several phthalates in the mothers urine as an indication of exposure to the foetus from the mother's body. To monitor whether phthalates impacted on male reproductive health, a measure was made of the anogenital distance in 134 male children aged 2-36 months. The anogenital distance is defined as the distance from the anus to the base of the scrotum which, in rodents, is known to be a sensitive measure of prenatal exposure to anti-androgens, the distance decreasing with increasing exposure. Assessment of the human male children revealed that, in accordance with animal studies, higher exposure to phthalates in the womb was associated with reduced anogenital distance. Higher exposure was also associated with impaired testicular descent. These results support the hypothesis that phthalate exposure in humans at current environmental levels may be adversely affecting male reproductive health. In other words, phthalate exposure could be one contributory cause of testicular dysgenesis syndrome. Further research will be necessary to confirm the findings (Sharpe 2005, Swan *et al.* 2005).

Impacts of mixtures of chemicals on male reproductive health was discussed at a recent conference called by the Committee on Toxicity, a body which advises the UK government on health impacts of environmental chemicals (ENDS 2006). Results of a yet unpublished study were given at the meeting. In this research, pregnant rats were given mixtures of chemicals which can have anti-androgenic effects. These included the phthalates DBP, DEHP and BBP and four pesticides. The study showed that the exposure to mixtures of the chemicals resulted in additive impacts on the male reproductive system of the rodent offspring. The results may explain how exposure to a cocktail of environmental chemicals, each at low levels, could have implications for male reproductive health.

2.1.3 Other possible causes of sperm count decline

A recent study in the USA investigated whether air pollutants were having an impact on sperm quality (sperm concentration and motility) (Sokol *et al.* 2006). The study found that increasing ozone concentration was associated with reductions in sperm quality and this indicated that ozone may be a reproductive toxicant. Ozone is the major oxidant present in low-altitude photochemical smog, a common phenomenon in the urban environment. It is thought that the mechanism by which ozone may exert its effect on sperm quality is through oxidative stress, a mechanism already known to disrupt testicular and sperm function. Cigarette smoking can cause a modest decrease in sperm concentration which is associated with oxidative stress, although no studies have looked at the overall association between cigarette smoking, pollution and semen quality.

Over the years, numerous other possible contributing factors have been proposed to explain observed declines in human sperm counts and quality, including dietary changes, lifestyle factors and even tight clothing. While the influence of such factors, individually or in concert, certainly cannot be ruled out, this does not detract from the parallel evidence indicating that exposure to hazardous, man-made chemicals may also be contributing to the decline, nor from the urgency therefore to address such exposures.

2.2 Female Reproductive Health

Over the past 50 years, women in industrialised countries have experienced a rise in abnormalities of the reproductive system. More women are suffering from endometriosis, osteoporosis continues to be a problem, and in some countries girls are entering puberty earlier.

2.2.1 Endometriosis

Endometriosis is a condition in which endometrial tissue, the tissue that normally lines the inside of the uterus, grows outside the uterus and attaches to other organs, usually in the pelvic cavity, such as the ovaries and fallopian tubes. This tissue is under normal hormonal control, and builds up, breaks down, and bleeds like the lining of the uterus. This internal bleeding into the pelvic or abdominal cavities, has no way of leaving the body, and leads to inflammation, pain and the formation of scar tissue. Endometrial tissue may also be found in the ovary where it can form cysts. Endometriosis causes intense chronic pain. It is estimated that one in ten women in the USA suffer from the disease, forcing more than 100,000 to undergo hysterectomies (removal of uterus) each year (Rier 2002).

A number of synthetic chemicals are known to increase the prevalence and severity of endometriosis although it is not known whether such chemicals are responsible for the rise in endometriosis in the general population. For example, in monkeys, PCBs and dioxins cause endometriosis and make it worse in animals that already have it. Other research suggests that PCBs and dioxins could increase the risk of endometriosis in women. People are regularly exposed to dioxin levels significantly above those that are known to cause endometriosis in monkeys (Rier 2002).

One study found that women with endometriosis have a higher level of the phthalate DEHP in their blood than normal women. Furthermore, 92.6% of these women also had detectable DEHP and/or the metabolite MEHP in their peritoneal fluid (see Table 2.1). This suggests that DEHP may play a role in causing endometriosis (Corbellis *et al.* 2003).

TABLE 3: LEVELS OF DEHP IN WOMEN'S BLOOD

GROUP	COMMON USES	REPRODUCTIVE HEALTH CONCERNS
Endometriosis patients	0-3.24 ug/ml	0.57 ug/ml
Normal Females	0-1.03 ug/ml	0.18 ug/ml

Source: Corbellis *et al.* (2003). ug = microgrammes

2.2.2 Earlier Puberty

In some parts of the world, girls are reaching puberty far younger than in the past, sometimes starting sexual development several years earlier than normal. A study in 2001 of children immigrating to Europe from parts of Latin America and Asia found that some girls started developing breasts before the age of eight and began their periods before they were ten (Krstevska-Konstantinova *et al.* 2001). Although scientists are unsure why this shift is occurring, exposure to the insecticide DDT in early life prior to immigration is one possible cause; high levels of the DDT breakdown product, DDE, were found in the blood of three-quarters of immigrating children exhibiting early puberty. DDT is banned in the developed world, but many countries in the South and East continue to use it against malaria-carrying mosquitoes. The pesticide is thought to have its effect because DDE mimics female oestrogen hormones.

A study of the distribution of early puberty in boys and girls in Tuscany, Italy, suggested that environmental factors, possibly pollution from endocrine disruptors, could be responsible for the more elevated incidence of early puberty in some areas (Massart *et al.* 2005).

A study of Puerto Rican girls also found an increasing incidence of premature breast development before the age of eight (thelarche), suggesting that the condition may be linked to elevated exposure to phthalates (Colon *et al.* 2000). Significantly high levels of phthalates were detected in blood samples from 28 out of 41 (68%) of the girls with thelarche. By comparison, high levels of one phthalate were detected in only 1 out of 35 blood samples from girls with normal breast development. The study suggested there was a possible association between phthalates with known oestrogenic and anti-androgenic activity and the cause of premature breast development. It was noted that in Puerto Rico there is high importation of plastic packaged foods, which, in combination with other factors, could contribute to higher overall phthalate exposure. A possible drawback of this study was that the timing of exposure to phthalates was unknown and the high exposure levels found may have reflected contamination of the blood samples by phthalates (Swan *et al.* 2005).

reproductive health trends and chemical exposure:
making the link *continued*

2.2.3 Other Reproductive Effects

A significant association between musk xylene and musk ketone levels in women's blood and hormonal and gynaecological problems has been shown in another study, suggesting that these musks may cause reproductive toxicity and endocrine effects in humans (Eisenhardt *et al.* 2001). Once again it has not yet been possible to determine whether or not this is a cause-effect relationship.

2.3 Changing Sex Ratio

The sex of an individual is genetically determined and the ratio of the number of boys born to the number of girls should in theory be roughly equal. In reality, there is a very slight excess of boys born in relation to girls, a fact which may be accounted for by several different factors including age of parents and the time of insemination within the cycle (Moller 1996).

However, evidence is emerging which indicates that the sex ratio, the number of boys compared to girls, is changing. The number of boys relative to girls is in decline (Allan *et al.* 1997, Davis *et al.* 1998, Vartianen *et al.* 1999). For example, the USA 'lost' an estimated 38,000 boys between 1970 and 1990. A similar trend began around 1950 in the Netherlands and Denmark. Since then, the USA, Canada, Sweden, Germany, Norway, Japan, Finland, and Latin America have all seen shifts in the ratio of boys to girls. In parts of Canada, nearly six boys per thousand are 'missing'. In some rural parts of the USA, three girls are born for every two boys.

It is possible, although not proven, that synthetic chemicals may be involved in the decline of the male birth rate by selectively killing male fetuses. Research published in the Lancet indicates that male fetuses are dying in unprecedented numbers in the first few months after conception, and they are doing so at ever-earlier stages of development (Mizuno 2000). This could explain, at least in part, the disappearing boys. The Lancet recorded that in 1966, 2.52 male fetuses died for every female between the ages of 12–15 weeks. In 1986 this had risen to 6.16. By 1996, more than ten male fetuses were dying for every female. In total, throughout pregnancy, more than twice as many male fetuses are dying as females.

The cause of the decreasing number of boys relative to girls is unknown but could possibly be linked to exposure of the general population to chemicals. For instance, a paper in the Canadian Medical Association Journal warns that:

"Exposure to environmental toxins has been shown to alter the sex ratio of live births in both human populations and animal models... It is possible that certain biological markers such as sex ratio and semen quality are being altered by as yet unidentified factors that may include environmental toxins" (Allan* et al. *1997).

Endocrine disrupting chemicals could potentially be to blame but there is no conclusive proof. One study on sex ratio changes in the Netherlands concluded:

***"Our findings of a decreasing ratio of male to female newborn babies in The Netherlands can only add to concern about the potential hazards of environmental endocrine disruptors"* (van der Pal-de Bruin et al. 1997).**

Contrary to this warning, one recent study on trends in sex ratio from 1960 to 1996 in California, did not find a change in sex ratio was likely to be due to endocrine-disrupting chemicals. The study found differences between different races such that there was a decline in males born to white people but not other races. Closer examination of the results suggested that any changes in sex ratio detected in this study were likely to be attributable to changing ethnic composition and were less likely due to exposure to endocrine-disrupting chemicals (Smith and Von Behren 2005).

Clearly, as in any epidemiological data set, the underlying cause-effect relationships are undoubtedly extremely complex and multi-faceted and are likely, therefore, to remain impossible to define accurately for the foreseeable future. Once again, however, the possible contribution from hazardous chemical exposure cannot be dismissed and demands close scrutiny.

other lines of evidence: direct measures of reproductive toxicity and chemical exposures

The hypothesis that chemicals we are exposed to every day may be causing problems in our reproductive health arises not only from the observations and correlations discussed above. The results of laboratory studies which have demonstrated the capability of various widely used chemicals to interfere with the endocrine systems or with reproductive development in other ways also provide an important line of evidence, as do direct measurements of the presence of those chemicals in body tissues of wildlife and humans.

Evidence of exposure of the developing child to chemicals comes from studies that have identified chemicals in the mothers' blood, in umbilical cord blood and tissues and in breast milk. In addition, young children can consume several times more food and water in proportion to their body size than adults and, consequently, ingest more chemicals per unit of body mass during the first years of their lives.

Using the same six chemical groups listed above as examples, this section expands on the results of laboratory studies of endocrine disruption and reproductive toxicity in animals or in cell culture systems and reviews the evidence for the presence of these chemicals in the human body, with particular emphasis on exposure in the womb.

3.1 Alkylphenols

3.1.1 Effects

Both nonylphenol and octylphenol show oestrogenic and anti-androgenic activities (Lee *et al.* 2003a; Paris *et al.* 2002). Alkylphenols have been shown to cause oysters and zebrafish to change sex (Nice *et al.* 2003).

Two studies on reproduction and development in rodents following low dose exposure to alkylphenols are reported in the scientific literature. Sharpe *et al.* (1995) showed that pre- and postnatal exposure to octylphenols caused a decrease in testicular size and daily sperm production in rats during a relatively short period. A multigenerational mouse study demonstrated that nonylphenol affected both the parents and offspring (Kyselova *et al.* 2003) with the predominant effects being on the size of male reproductive organs, sperm quality and fertility.

An *in vitro* study which used cells taken from human fetal gonads showed that 4-octylphenol affected the rate of proliferation of the germ cells and may therefore interfere with testicular function (Saradha and Mathur 2006).

3.1.2 Exposure

Despite increasing restrictions on their use in Europe, alkylphenols remain widespread as contaminants in our environment, including in our food. For example, a study of 60 food-products on the market in Germany illustrated the widespread nature of alkylphenol contamination (Guenther *et al.* 2002). These were all popular, common foods in Germany, including 39 adult foods, from marmalade to sausages, 20 baby foods, and one sample of breast milk (see Table 4 for a selection of the results). Nonylphenol was detected in every sample within the range of 0.1–19.4 microgrammes/kg, and was not related to fat content. The authors stressed that, since the foods varied substantially in nature, source, preparation methods and packaging, it is likely that there are multiple entry points for nonylphenol into the human food supply.

TABLE 4: EXAMPLES OF NONYLPHENOL LEVELS IN INFANT FOODS, AND GENERAL FOODS THAT MAY BE FED TO INFANTS.

FOOD	NONYLPHENOL LEVELS (ug/kg)
Breast milk (35-year old mother)	0.3
Infant formula	11.6
Infant formula	22.1
Banana & milk puree	0.2
Peach & honey puree	0.4
Carrots puree	0.8
Semolina & vanilla puree	1.8
Broccoli, potato, turkey puree	1.4
Beef, potato & rice puree	3.1
Noodles in ham & tomato sauce	4.0
Peach & passion fruit yoghurt	0.6
Whole milk (3.5%)	0.4
Evaporated milk (10%)	3.8
Hen's egg	1.5
Tuna	8.1
Apples	19 .4
Orange juice	0 .1

Source: Guenther *et al.* 2002. ug = microgrammes

other lines of evidence: direct measures of reproductive toxicity and chemical exposures *continued*

There have been few studies on levels of human contamination by alkylphenols, but those that have been performed clearly show that children are contaminated before birth (umbilical cord) and after birth (breast milk) (see Table 5). Nonylphenol has been detected in human umbilical cords (Takada *et al.*1999), confirming that it crosses the placenta from the contaminated mother to the growing foetus. This was more recently reaffirmed by the joint Greenpeace/WWF study into chemical contaminants in human umbilical cord blood donated by volunteer new mothers in the Netherlands (Greenpeace/WWF 2005). Nonylphenol itself was detected in 12 of the 17 cord blood samples analysed in this study.

TABLE 5: HUMAN CONTAMINATION BY NONYLPHENOL

SOURCE	LEVELS	REFERENCE
Umbilical cord	2 ng/kg wet tissue	Takada *et al.*1999
Breast milk	0.3 mg/kg lipid	Guenther *et al.* 2002

3.2 Phthalates (phthalate esters)

3.2.1 Effects

Phthalates, more specifically diesters of phthalic acid, are generally considered to be 'weak' hormone disrupters that can act as oestrogens but also, perhaps more potently, as anti-androgens. Testicular toxicity is partly caused by interference with binding of follicle stimulating hormone (FSH) to its receptor on Sertolli cells, the cells involved in sperm production (Boiter *et al.*2003). Much of the toxicity relating to phthalates in the body may actually be caused by the primary metabolites or breakdown products of the original phthalate diester compounds, the so-called monoesters.

Phthalates may also bind to the oestrogen receptor and either mimic oestrogen hormones or reduce their effectiveness. Those with anti-oestrogenic activity are more commonly the monoester forms. For example, a study of the effects of 19 phthalates or phthalate metabolites in human breast cancer cells (Okubo *et al.* 2003) showed that:

* dicyclohexyl phthalate (DCHP), bis(2-ethylhexyl) phthalate (DEHP) and benzylbutyl phthalate (BBP) are oestrogenic
* monomethyl phthalate (MMP), monocyclohexyl phthalate (MCHP), monobenzyl phthalate (MBzP) and monoisopropyl phthalate (MIPrP) are anti-oestrogenic.

Following conversion to the monoester forms, phthalates can cause foetal death, malformations, and reproductive toxicity in laboratory animal studies, with different potencies and effect profiles for each phthalate. Maternal exposure of rodents to DEHP / MEHP caused reduced embryonic implantation, increased resorptions, intrauterine death and increased postnatal death in rodent pups (Gray 2000, Moore *et al.* 2001). Foetal toxicity can occur without evidence of maternal toxicity.

The immature male reproductive tract still appears to be the most sensitive system. Pathological changes in the testis and decreased sperm numbers are commonly recorded effects of phthalate exposure in animals. Other changes include reduced anogenital distance, cleft phallus, hypospadias and undescended testes (Fisher 2004). Prenatal and postnatal exposure leads to complete female infertility and decreased male fertility. Sertoli cells in the testicle and the granulosa cells of pre - ovulatory follicles in the ovary appear to be the primary cellular target of DEHP/MEHP.

Other phthalates appear to have a similar pattern of toxicity but at higher doses. For example:

* DBP is a testicular toxicant and causes reproductive tract malformations in male rats after *in utero* exposure (Lovekamp-Swan & Davis 2003).
* Maternal exposure to low doses of BBP (125–370 microgrammes/kg/day) lead to decreased testes weight in male offspring following prenatal and postnatal exposure (Sharpe *et al.* 1995).
* DPP and DHP cause testicular atrophy and are both female and male reproductive toxicants (Lovekamp-Swan & Davis 2003).

3.2.2 Exposure

Phthalate exposure is widespread and continuous, a result of their high volume use in PVC and other open applications, which has led to them becoming one of the most ubiquitous man-made chemicals in our environment. Our exposure to them can result from leaching from products such as soft PVC (vinyl) flooring, furnishings, clothing etc. as well as through inhalation of contaminated indoor air, exposure to household and/or office dusts, consumption of contaminated food or, in some cases, of contaminated drinking water. Concentrations in house dust can reach several milligrammes per gramme of dust (parts per thousand). The use of phthalate esters in products such as perfumes, which may contain high levels in particular of the phthalate DEP as a solvent and alcohol denaturant (Greenpeace 2005), may result in additional exposure.

With respect to food intake, presence of phthalates in food contact materials may be of particular concern. In one study, the simple process of frying and packaging chicken increased the phthalate DEHP content from 0.08 to 16.9 mg/kg, with the main source of contamination thought to be PVC gloves used by food workers (Tsumura *et al.* 2001b).

More recently, a study published by Greenpeace offices in Central and Easter Europe documented the presence of a range of phthalate esters in the flesh (muscle tissue) of farmed carp purchased from supermarkets from Austria, Czech Republic, Poland and Slovak Republic (Greenpeace CEE 2005). Levels of several phthalates, especially DiBP (diisobutyl phthalate), DBP and DEHP, were unexpectedly high in the fish, though it is not clear whether the majority of this contamination arises from the environment in which the fish are reared or from materials used in the processing and packaging of the fish before they are sold.

PVC tubing has in the past also been a source of high-level contamination of baby food (Tsumura *et al.* 2001a).

Metabolites of phthalates are detectable in urine samples from adults indicating exposure to phthalates (Barr *et al.* 2003, CDC 2003, Koch *et al.* 2003). Animal studies show that phthalates cross the placenta and pass into breast milk (Dostal *et al.* 1987; Parmar *et al.* 1985; Srivasta *et al.* 1989). One study on humans detected six phthalate monoesters in breast milk, namely mono-methyl phthalate (mMP), mono-ethyl phthalate (mEP), mono-n-butyl phthalate (mBP), mono-benzyl phthalate (mBzP), mono-2-ethylhexyl phthalate (mEHP), mono-isononyl phthalate (miNP) (Main *et al.* 2006). Their ability to cross the human placenta and therefore reach the developing child in the womb was confirmed by the common occurrence of phthalate esters, especially DEHP, DBP, BBP and DEP, in samples of umbilical cord blood (Greenpeace/WWF 2005). DEHP was by far the most common, appearing in 24 of the 27 samples of cord blood analysed at concentrations up to several parts per million in the cord blood serum.

Children may be more exposed to phthalates than adults. For example, in one study, of the seven urine phthalate metabolites tested, the highest levels of metabolites for DEHP, DBP and monobenzylphthalates were found in the youngest age group tested: the 6–11-year-old children (CDC 2003).

The phthalate di(2-ethylhexyl) phthalate (DEHP) is used as a plasticiser in medical products made of PVC, for example, feeding tubes, and may leach out from such products. Particularly high exposures to DEHP, the only phthalate currently used to plasticise medical devices, can occur during medical interventions (EC 2002).

One study has assessed whether the use of such medical products results in exposure of newborns receiving treatment in neonatal intensive care units (Green *et al.* 2005). The study monitored the level of mono(2-ethylhexyl) phthalate (MEHP), a metabolite of DEHP, in 54 babies potentially exposed to DEHP through medical equipment. Results showed that intensive use of DEHP-containing medical devices resulted in higher exposure to DEHP, reflected in elevated levels of MEHP in urine.

other lines of evidence: direct measures of reproductive toxicity and chemical exposures *continued*

3.3 Brominated Flame Retardants

3.3.1 Effects

A range of PBDEs and brominated bisphenol A compounds, such as TBBP-A, show oestrogenicity in human cells lines and bind to the oestrogen receptors (Meerts *et al.* 2000). The metabolism of PBDEs to hydroxylated-PBDEs produces more potent oestrogen mimics. Brominated bisphenol A compounds with the lowest bromination showed the highest effect, and among the PBDEs, BDE-100, BDE-75 and BDE-51 showed the highest activity (Olsen *et al.* 2003).

Toxicity studies of BFRs in animals are limited and primarily consist of high dose studies of PBDEs. Nevertheless, there is evidence that chronic exposure to PBDEs can cause birth defects in rodents (Darnerud *et al.* 2001, Darnerud *et al.* 2003), as well as impacts on nervous system and behavioural development.

3.3.2 Exposure

PBDEs may have a similar range of exposure sources as the phthalates. Food is almost certainly the main source of exposure to some of the more bioaccumulative (lower brominated) PBDEs. PBDEs have been detected in fish and shellfish in the range of 21–1650 pg/g fresh weight (Ohta *et al.* 2002). In comparison, beef, pork, and chicken contained 6.25–63.6 pg/g, and three different vegetables had levels of 38.4–134 pg/g. Ohta *et al.* (2002) showed a strong correlation between PBDE levels in breast milk and intake of fish and shellfish. For chemicals such as decabromodiphenyl ether (BDE-209 or 'deca'), however, more direct exposure to e.g. contaminated indoor dusts or even direct contact with products may be relatively more significant in terms of human exposure.

'PDBEs have been found in human breast milk, blood, and adipose tissue (eg. Hardell *et al.* 1998, Schroter-Kermani 2001, Guvenius *et al.* 2003). Levels of PBDEs in breast milk and umbilical cord are given in table 6. Extensive breast milk studies in Sweden show an exponential increase in PBDEs in breast milk (an average increase from 0.07 to 4.02 ng/g lipid between 1972–1997) (Meironyte *et al.* 1999). However, a recent paper has reported a decrease of PBDEs in Swedish breast milk since 1997, possibly due to a voluntary phase out of penta-BDE (Hooper & She, 2003).

Babies born to mothers in the USA may be more at risk of PBDE contamination than in Sweden and Norway. Mazdai *et al.* (2003) found that the concentrations of PBDEs in maternal and foetal serum samples in Indianapolis, USA, were 20–106 times higher than the levels reported previously in Swedish mothers and infants (Guvenius *et al.* 2003) and 20 times higher than Norwegian blood samples (Thomsen *et al.* 2002).

TABLE 6: HUMAN CONTAMINATION BY POLYBROMINATED DIPHENYL ETHERS

PBDE	SOURCE	LEVELS	REFERENCE
PBDEs (8 identified including BDE-47	Breast milk	4.02 ng/g lipid (mean)	Meironyte *et al.* 1999
PBDEs	Breast milk	0.67–2.84 ng/g lipid	Ohta *et al.* 2002
PBDEs	Breast milk	75.0 pg/g fresh weight	Guvenius *et al.* 2003
PBDEs	Umbilical cord blood	4.3 pg/g fresh weight	Guvenius *et al.* 2003
PBDEs (6 including BDE-47)	Umbilical cord blood	14-460 ng/g lipid	Mazdai *et al.* 2003

3.4 Organotin compounds

3.4.1 Effects

Organotins are hormone disrupters probably best known because of the devastating effects of tributyl tin (TBT) used in antifouling paints on certain marine molluscs. However, both TBT and the pesticide triphenyl tin (TPT) have been observed also to inhibit a variety of enzymes responsible for the production of male and female sex steroid hormones in other organisms, including testosterone and oestradiol (Doering *et al.* 2002, Lo *et al.* 2003, Steckelbroek *et al.* 2001).

While low-dose developmental studies are lacking in mammals, insufficient activation of male hormones is known to be responsible for developmental disorders of the male reproductive tract. A recent study suggested that organotins may also cause developmental effects *in utero* at relatively low doses by targeting the maternal thyroid (Adeeko *et al.* 2003). Effects varied depending on dosage but appeared to be linked to the reduction of maternal serum thyroxine and triiodothyronine throughout gestation. Effects included reduced maternal weight gain, increased post-implantation loss, decreased litter sizes, decreased foetal weights, delayed foetal skeletal development, and abnormalities in genital development in male fetuses.

3.4.2 Exposure

The presence of TBT in seafood, primarily a result of its former use as an antifouling agent in ship hull paints, has led, in some regions, to elevated intakes. In Japan, for example, where fish is a major part of the diet, the estimated daily intake of the organotin TBT is relatively high (2.5 microgrammes/kg body weight based on a fish consumption of 150 g/day) (van Heijst, 1994).

At the same time, we may be exposed to TBT and other organotin compounds, including the mono- and dibutyl forms (MBT and DBT) used *inter alia* as stabilizing additives in PVC, from a range of other, sometimes rather unexpected, sources. For example, TBT, DBT and MBT have all been reported to leach from some brands of baking parchment, and DBT and MBT from gloves for kitchen work, dish-washing sponges and cellophane film for food, on sale in Japan (Takahashi *et al.*1999).

Although organotins, particularly TBT, have been reported in a wide range of molluscs, fish, marine birds, marine mammals, and freshwater birds (IPCS, 1999), aside from a few reports, levels of contamination in humans are largely unknown, and there are no readily available reports on child contamination.

TABLE 7: ASSUMED SAFE DOSE OF BISPHENOL A COMPARED TO EVIDENCE OF LOW-DOSE TOXICITY IN RODENTS

EFFECTS	DOSE (mg/kg/day)	REFERENCE
Assumed safe dose for animals	5.0	US EPA 1993
*Assummed safe dose for humans**	0.05	US EPA 1993
Effects on vagina	0.1	Schonfelder *et al.* 2002a
Increased prostate size	0.05	Gupta *et al.* 2000
Long-term alterations in behavioural patterns in adolescence and adulthood	0.04	Adriani *et al.* 2003
Abnormal prostate development	0.025	Ramos *et al.* 2001 and 2003
Abnormal mammary gland growth	0.025	Markey *et al.* 2001
Reduced sperm production	0.02	Vom Saal *et al.* 1998, Sakaue *et al.* 2001
Early puberty in females	0.02	Honma *et al.* 2002
Altered maternal care	0.01	Palanza *et al.* 2002
Early puberty in females	0.0024	Howdeshell *et al.* 1999
Altered male reproductive glands	0.002	vom Saal *et al.* 1998
Increased adult prostate weight	0.002	Nagel *et al.* 1997 and 1999
Reduced testis weight	0.002	Kawai *et al.* 2003

*The oral Reference Dose (RfD) is an estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

other lines of evidence: direct measures of reproductive toxicity and chemical exposures *continued*

3.5 Bisphenol A

3.5.1 Effects

Bisphenol A binds to oestrogen receptors range of human cell lines and mimics all the oestrogenicity parameters, confirming it as one of the stronger oestrogenic chemicals (Meerts *et al.* 2000, Olsen 2003).

The health effects of bisphenol A have been demonstrated in an ever- increasing number of animals studies at levels up to 2,500 times lower than the EPA's 'lowest observed dose effect' dose. The adverse outcomes range from altered male reproductive organs and aggressive behaviour, to abnormal mammary gland growth, early puberty and reduced breast feeding (Table 7). Induction of early puberty in laboratory animals can occur at extremely low doses (Howdeshell *et al.* 1999).

Human infants ingest bisphenol A in formula at an estimated daily rate of 1.6 microgrammes/kg/day, giving little safety margin for doses that cause effects in animals (as little as 2 microgrammes/kg/day) (Houlihan *et al.* 2003).

3.5.2 Exposure

While there are many potential exposure routes, the principal concern regarding exposure to bisphenol A and its derivatives remains contamination of food. Bisphenol A can leach into food as a result of the presence in food contact materials of un-reacted compound as well as from degradation of polymers such as polycarbonate (typical plastic used to make baby bottles). It has been found to migrate from rubber products and plastic stretch-film used in food-contact applications (Lopez-Cervantes & Paseiro-Losada, 2003, Ozaki & Baba, 2003), as well as from the lining of many food cans. Bisphenol A levels migrating from plastic products into baby food increase after dishwashing, boiling, and brushing (Brede 2003).

In some instances, contamination has even been reported to arise from water filters (Inoue *et al.*2000). Furthermore, patients on kidney dialysis may receive elevated exposures as a result of the use of polycarbonate components in the equipment (Yamasaki *et al.* 2001)

The subject of the effects of bisphenol A on humans has been contentious. It has been suggested that it is only partially absorbed, has a high conversion rate to the biologically inactive bisphenol A monoglucuronide, is rapidly excreted, and shows no evidence of bioaccumulation in tissues (Schonfelder *et al.* 2002b). For these reasons, until recently, many scientists believed that the active parent form of bisphenol A would not be found in human plasma, and therefore no significant levels could reach the foetus.

However, studies from Germany and Japan have now confirmed that children are exposed to bisphenol A before birth. The first, a Japanese study, found bisphenol A in umbilical cords (Takada *et al.*1999). Studies on mice and monkeys then showed that this chemical can cross the placenta (Uchida *et al.* 2002). Other studies have also reported the presence of bisphenol A in umbilical cord blood (Table 8).

Data from Ikezuki *et al.* (2002) suggest that bisphenol A may concentrate in amniotic fluid as it was found at approximately 5-fold higher concentrations at 15–18 weeks gestation, compared with other fluids. Schonfelder *et al.* (2002b) also showed that in 14 of 37 cases, foetal plasma levels of bisphenol A were higher than in the corresponding mother's blood. Foetal levels of bisphenol A were also significantly higher in males, which may indicate sex differences in the metabolism of this chemical. Takeuchi and Tsutsumi (2002) also found this gender difference in a study on adults, and suggested that it may be due to androgen (male hormones) related metabolism of bisphenol A.

TABLE 8: HUMAN CONTAMINATION BY BISPHENOL A

SOURCE	LEVELS	REFERENCE
Umbilical cords	1.6 ng/g wet tissue	Takada *et al.* 1999
Umbilical cord blood	2.9 ng/ml (median) 0.2-9.2 ng/ml	Schonfelder *et al.* 2002b
Umbilical cord blood	0.62 +/- 0.13 ng/ml (mean)	Kuroda *et al.* 2003
Amniotic fluid at 15-18 weeks	8.3 +/- 8.7 ng/ml (mean)	Izezuki *et al.* 2002
Amniotic fluid	0.26 ng/ml (median)	Yamada *et al.* 2002

3.6 Artificial Musks (nitromusks and polycyclic musks)

3.6.1 Effects

Artificial musks are persistent and bioaccumulative chemicals. Musk xylene (MX) and musk ketone (MK) possess oestrogenic activity in vitro with MK showing an affinity for the oestrogen receptor three times greater than MX (Bitsch *et al.*2002). However, when MK is reduced to its metabolite it loses its activity, whereas when MX is converted to p-amino-musk xylene, its oestrogenic potency increases (Bitsch *et al.* 2002).

The polycyclic musks, AHTN and HHCB, induce both oestrogenic and anti-oestrogenic activity depending on the cell type and the receptor subtype targeted. Weak oestrogenic effects are observed at relatively high concentrations (10 micromolar) while anti-oestrogenic effects are seen at 0.1 micromolar (Schreurs *et al.* 2002), including effects in whole organisms.

3.6.2 Exposure

Although historically nitromusks (including MX and MK) dominated the European market for fragrance additives, their place has since been taken by the polycyclic musks, especially AHTN and HHCB. Their use in cleaning and personal care products is thought to remain widespread, though few product-specific data exist. In what remains one of the few available studies, Greenpeace published in February 2005 a report quantifying the presence of a range of synthetic musk compounds, including AHTN and HHCB, in perfumes sold in Europe (Greenpeace 2005).

Nitromusks have been found in adult human adipose tissue and blood (Rimkus and Wolf 1996, Kafferlein and Angerer 2001). Several studies have reported the presence of these chemicals in breast milk (table 9). In addition, both HHCB and AHTN were frequently found in samples of human umbilical cord blood in the Netherlands (Greenpeace/WWF 2005), albeit at low ppb levels in the serum, with HHCB detected in all but one of the 27 samples analysed.

TABLE 9: HUMAN CONTAMINATION BY ARTIFICIAL MUSKS

MUSK	SOURCE	LEVELS	REFERENCE
xylene	Breast milk (n=391)	100 ug/kg fat (mean)	Liebl and Ehrenstorfer 1993
ketone		40 ug/kg fat (mean)	
tonalide	Breast milk (n=5)	8-58 ug/kg fat	Rimkus and Wolf 1996
mosken	Breast milk (n=53)	64 ug/kg fat (mean)	Zehringer and Hermann 2001
tibeten		25 ug/kg fat	
Xylene		35 ug/kg fat	
HHCB		73 ug/kg fat	
AHTN		44 ug/kg fat	
Traseolide		74 ug/kg fat	

ug = microgrammes

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conclusions

* The body of evidence for increases in reproductive disorders in humans is growing and should be reason for serious concern about the future ability of mankind to reproduce.
* Exposure during early stages of life is of particular concern. Both unborn and newborn babies are thought to be more susceptible to chemical exposure. Hormones play many critical roles in controlling growth and development in early life, such that any interference could have serious and irreversible effects on child development with consequences that may be felt throughout their later lives. Some diseases and other health conditions can develop many years or even decades after the key period of chemical exposure; even though the damage may be done at a very young age, the health consequences may not be realised until adolescence or adult life.
* Many man-made chemicals, often with hazardous properties, are produced and used every day in Europe and elsewhere. This results in large scale contamination of the environment and human bodies by some of these chemicals.
* Numerous widely used man-made chemicals, including some used as additives in products we make use of every day, have shown toxicity to reproduction and/or hormone disrupting properties in laboratory studies.
* There is also an increasing body of research documenting the presence of hazardous chemicals in humans (e.g. in blood and body tissues) and the findings of correlations (statistically significant associations) between level of exposure and incidence of certain disorders.
* In short, although not proven beyond doubt, there is increasing evidence of a possible link between the synonymous rise of reproductive health problems in humans and the rise of our exposure to many chemicals. The presence of many man-made chemicals at current environmental levels may already be negatively impacting the reproductive health of wildlife and humans.

Despite these concerns, many chemicals with known or suspected toxicity to the reproductive or hormone systems remain in use. Furthermore, the full scale of the problem is not known as there is still a substantial lack of information even about basic properties of most of the chemicals commonly manufactured, sold and used in Europe today. The EU's REACH proposal should therefore strengthen its requirements for data to be provided during the registration process, so that even low tonnage chemicals could be evaluated for their potential reproductive toxicity and hormone disrupting properties.

In reality, our exposure to hazardous chemicals is multiple and highly complex. Knowledge about the effects of mixtures, or "cocktails", of chemicals, which can occur even at relatively low doses, remains very limited.

In such highly complex systems as our environment and our bodies, regulatory approaches which attempt to establish 'safe' doses or 'acceptable' risks and thereby 'manage' exposure, to chemicals suspected or known to harm fertility or the unborn child, will inevitably be unable to guarantee a high level of protection for the environment and human health. For such chemicals, the only sound approach would be to prevent exposure by establishing a goal to eliminate the manufacture and use of these chemicals wherever possible. This goal could be reached by requiring mandatory substitution of hazardous chemicals by safer alternatives, thus also driving innovation towards green chemistry.

The situation outlined in this report clearly indicates the need for precautionary action to prevent further exposure of humans and wildlife to hazardous chemicals, including those having impacts on reproduction. The proposed European REACH legislation has such potential if it is agreed in a form which will require sufficient information to be provided about the properties of chemicals before they can be sold or used, and mandatory substitution of the most hazardous chemicals, including CMRs (carcinogens, mutagens and chemicals toxic to reproduction) and endocrine disruptors.

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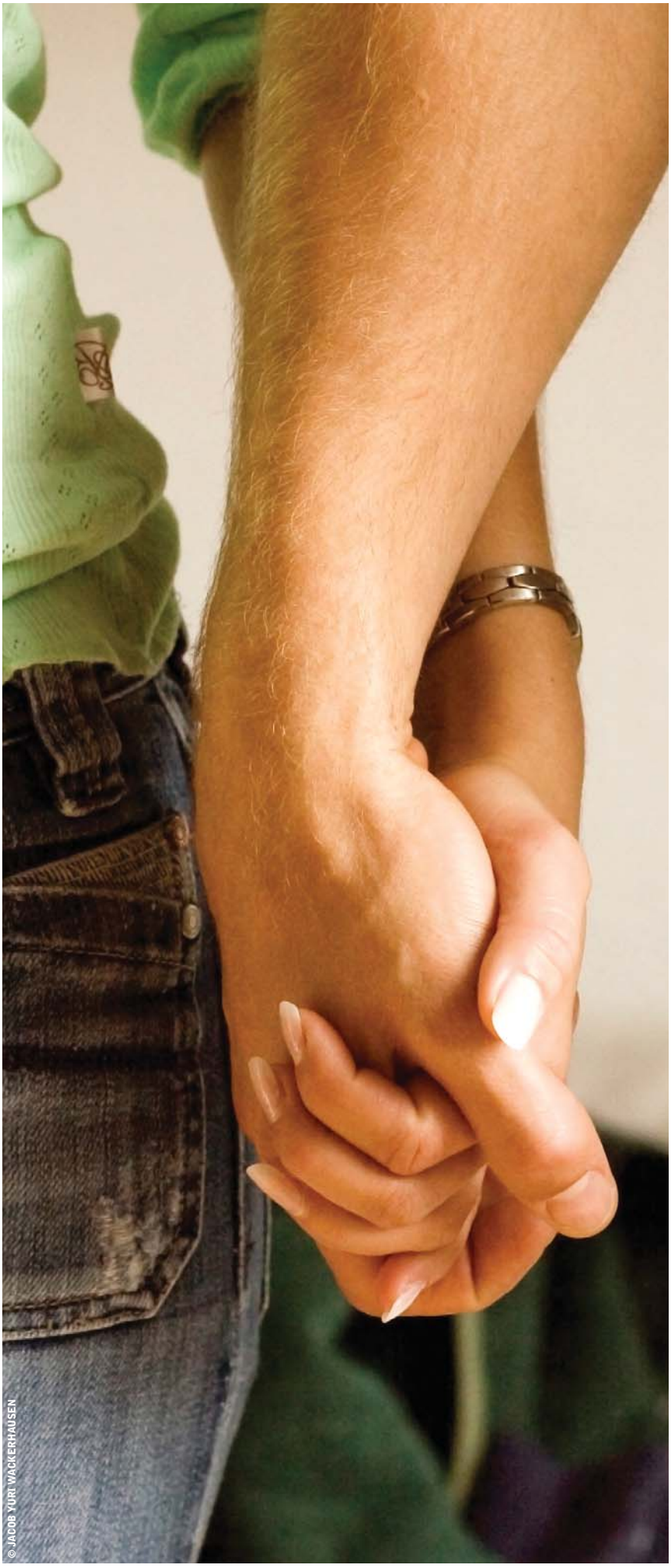

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