Bisphenol A

A Known Endocrine Disruptor

A WWF European Toxics Programme Report

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Introduction and overview

Bisphenol A (BPA) is an industrial chemical, used to manufacture polycarbonate and numerous plastic articles. However, recent studies have shown that it can leach out of certain products, including the plastic lining of cans used for food, polycarbonate babies' bottles and tableware, and white dental fillings and sealants.

Low levels of BPA have also been found to cause biological effects, and its mode of action appears to mimic that of the female hormone, oestrogen. BPA therefore belongs to a group of chemicals termed "hormone disruptors" or "endocrine disruptors", that are able to disrupt the chemical messenger system in the body. There is growing international concern about man-made endocrine disrupting chemicals (EDCs), because they can de-rail the development of offspring exposed in the womb. It is feared that they may be partly responsible for the decline in sperm counts, and the increased rates of hormone related cancers, such as cancers of the breast, testes and prostate. They are also suspected of causing birth defects of the reproductive tract (including un-descended testes), and other hormone related effects, such as earlier puberty in girls.

WWF is particularly concerned because the reproduction and development of many wildlife species have already been affected by EDCs released into the environment. WWF is therefore working to ensure that these substances are adequately controlled, which in many circumstances will include phasing them out of use. Even at very low levels, recent studies have shown that BPA can affect the sex ratio of frogs,[1] and can cause the sterilisation of water snails.[2] Given the intricate nature of ecosystems, and the lack of knowledge of the effects on many other species, this is a major concern.

With regard to possible effects in humans, alarm bells really started to ring in 1997, when one research team, fronted by Fred vom Saal and Wade Welshons, found that even very low levels of BPA could cause harmful effects. Male mice exposed in the womb to low levels of BPA were shown to have increased prostate weights,[3] and decreased daily sperm production.[4] Furthermore, these scientists found that BPA could cause greater effects at low dose levels than at higher doses. This launched the debate on the "low dose theory" or the inverted U-shaped dose response curve. As rodents are used to predict effects in humans these findings were critical.

Industry rushed to examine these low level effects of BPA, and several industry studies were published which could find no effects.[5,6,7] Subsequently, however, independent scientists working in the USA[8] and Germany[9] have confirmed that relatively low levels of BPA can indeed cause effects on male animals exposed in the womb. Industry scientists have argued that the divergence of results with regard to prostate weights, is due to the natural variability of the pups within a litter.[10] Whilst this may be so for some of these experiments, it is unable to explain the effects that have now been noted in female offspring and in young animals. For example, vom Saal's team have reported effects on female offspring, indicating that animals exposed to low doses may come to puberty earlier, and in this study the natural variability due to their position in the womb was fully taken into account.[11] Furthermore, other studies have reported changes in vaginal cells and the oestrous cycle in female mice exposed in the womb to relatively low levels of BPA.[12] Reported effects on young animals also underline the need to be

concerned about exposure to low levels. For example, a Japanese study has suggested a direct effect on sperm production in rats given a low dose for just 6 days.[13] Also, behavioural effects from early life exposures have now been identified.[14] Therefore, there are now several studies that strongly support the suggestion that BPA can cause effects at low doses, including effects on the female reproductive tract, and on breast tissue,[15] as well as effects on the testes and sperm production, and on the behaviour of both males and females.

The experience gained with BPA, and similarly with tobacco,[1] underlines the need to ensure that research scientists with no industry connections are properly resourced. It is notable that it is independent researchers who have highlighted possible concerns with regard to the effects of BPA exposure on human health and the environment.

This briefing provides background information on the production and use of BPA, and summarises the known exposure routes and research detailing the effects of BPA. Given the concerns about the effects of BPA and related substances, it is of paramount importance to identify and quantify all likely exposure routes.

This briefing also highlights some hitherto unpublished work, which suggests that in Europe, some polycarbonate babies feeding bottles may leach bisphenol A. This is clearly undesirable, particularly in view of the dose level which vom Saal considers may bring on earlier puberty in females. Male babies may also be at risk, as it would be a mistake to assume that hormonally speaking, babies are inactive. For example, during the first three months of life, male babies have high levels of male hormones (around 50% of adult levels).[16,17] It is not known exactly why this is, but it is believed that the subsequent behaviour of the individual is imprinted at this time.[18] Therefore, interference in hormonal processes at this age could have significant consequences to development.

The over-riding conclusion of this briefing is that current legal limits are not set at low enough levels to protect human health, and that human and wildlife exposure to BPA should be eliminated where practicable. In the interim, WWF considers that the public should be given access to all research findings, and regulators should aim to honestly inform the public about the concerns and uncertainties with regard to the effects of BPA exposure. Furthermore, the public should be given the right to know about the constituents of products, in order to enable them to make informed choices. This and other conclusions and recommendations are set out at the end of this briefing.

[1] It should not be forgotten, for example, that review articles written by authors with affiliations to the tobacco industry have been found to be 88 times more likely to conclude that passive smoking is not harmful than if the article is written by authors with no connection to the tobacco industry (Wise, J. (1998) BMJ. 316)

Production

Many countries throughout the world have large production capacities for BPA, especially Germany, the Netherlands, the USA, and Japan. Major companies include Dow, Bayer, Shell, GE Plastics, Aristech, Mitsubishi, Mitsui, and Shin Nihon.

TABLE 1: GLOBAL BISPHENOL A CAPACITY IN THOUSAND TONNES PER YEAR

(Taken from European Chemical News, 18-24 October 1999, but including modifications suggested by Vos of GE Plastics)

West Europe		
Bayer	Antwerp, Belgium	140
	Krefeld-Uerdingen, Germany	160
Dow	Stade, Germany	100
GE Plastics	Bergen op Zoom, Netherlands	210
	Cartagena, Spain	110
Shell	Pernis, Netherlands	110
East Europe		
Petro Borzesti	Borzesti, Romania	10
ZC	Blachownia, Poland	10
North America		
Aristech	Haverhill, Ohio	110
Bayer	Baytown, Texas	120
		160
Dow	Freeport, Texas	45
		166
GE Plastics	Burkville, Alabama	68
	Mount Vernon	260
Shell	Deer Park, Texas	102
		113
Asia		
Wuxi Resin	Wuxi, China	10
Kesar	Loteparhuram, India	7.5
Idemitsu	Chiba, Japan	70
Mitsubishi	Kashima, Japan	80
Mitsui	Nagoya, Japan	80
	Osaka, Japan	60
Shin Nihon (Mitsubishi Chemical/Nippon Steel Chemical)	Kyusu, Japan	95
Mitsui	Pulau Sakra, Singapore	70
Kumho P & B	Yeochon, S Korea	30
Nan Ya	Mailiao, Taiwan	72
Chang Chun	Mailiao, Taiwan	20
Taiwan Prosperity	Linyuan, Taiwan	25

In the EU alone, in 1997/98, annual consumption of BPA was estimated at approximately 640,000 tonnes (640 x 10^6kg) per year. EU manufacturers of BPA include: Bayer in Belgium and Germany; Dow in Germany; GE Plastics in the Netherlands and Spain; and Shell in the Netherlands.

Global production is reported to be increasing at about 7% per year, and to meet the increase in demand, Bayer is opening a new factory in Thailand. However, in 1999, Shell Chemical's global BPA business was up for sale.[19]

Uses

About 65% of the bisphenol A produced is used to make polycarbonate, and approximately 25% is used in epoxy resin production. The remaining 10% is used in other products such as speciality resins and in the manufacture of flame retardants, such as tetrabromobisphenol A.[20]

Bisphenol A is therefore used in the manufacture of a great variety of products including: compact disks, food can linings, thermal (fax) paper, safety helmets, bullet resistant laminate, plastic windows, car parts, adhesives, protective coatings, powder paints, polycarbonate bottles and containers (including returnable milk and water bottles) and the sheathing of electrical and electronic parts.[21] BPA is also used in PVC production and processing, where it may be used as a reaction inhibitor, and as an anti-oxidant.

Environmental exposures and effects on non-mammalian wildlife

Discharges to the environment occur not only from factories producing BPA, but also from numerous factories where BPA is incorporated into plastics or used in other products. Releases to the environment can also occur from sites where thermal fax paper is recycled, and possibly also from landfill sites. Discharges to air are expected to break down fairly rapidly, but discharges to water are likely to be more persistent and more of a problem.

There are very little data on the levels of BPA in the aquatic environment, but even at very low exposure levels BPA appears to affect the sexual differentiation of frogs. For example, two to three days after they had hatched, Kloas and co-workers exposed the tadpoles of frogs (*Xenopus laevis*) to BPA for 12 weeks. They found that at a BPA exposure level of 23µg/l there was a significant increase in the number of females, and that a concentration of 2.3µg/l BPA caused a smaller, but not significant, increase in the number of females.[22] Such an alteration in sex-ratios may have an important effect on population levels.

Other workers have shown that exposure to even lower levels of BPA can be harmful. For example, Oehlmann and colleagues have found that BPA, at levels down to 1µg/l, can cause dramatic effects in female freshwater ramshorn snails (*Marisa cornuarietis*). BPA apparently

stimulates egg production and causes swelling of the female sexual glands which results in blocked ducts. This blockage prevents the eggs from being transported, so that the egg-containing gland can be put under so much pressure that it bursts, and effectively these snails are sterilised. In some cases they also die because of the increased risk of infection.[23]

There is also concern about the possible effects on sediment dwelling organisms, because much of the BPA released to the water environment is expected to adsorb to sediments. However, there is currently a lack of knowledge about BPA's ability to degrade in anaerobic (un-oxygenated) sediments, and there is also lack of information on the potential effects on sediment dwelling organisms.

The effects at low exposure levels, coupled with possible concern about effects on sediment dwelling organisms, provide a powerful argument for the goal of eliminating environmental exposures.

Human Exposure

OVERVIEW OF HUMAN EXPOSURE

Human exposure can arise from a number of sources, particularly from the direct contact of food with BPA containing plastics. BPA leaching from the plastic material used to line food and drink cans has received particular attention. Other exposure routes that are a focus of attention include BPA leaching from babies' feeding bottles, and BPA and related compounds leaching from dental fillings and sealants. These sources are all dealt with in more detail below.

With regard to water pollution, a UK study has highlighted the potential for bisphenol- related substances to be present in drinking water via the materials used in the supply system. It underlines the need to control and reduce initial exposures from newly installed products, but the study was unable to accurately determine worst case exposures. Polycarbonates (made with BPA) and polysulphones (made with BPA and bisphenol S) are used to produce bottles for the storage of mineral waters, and so bottled water is also a potential source of BPA (see studies outlined below).[24] Wine stored in plastic bottles may also be contaminated to some extent.[25] With regard to raw water contamination, the potential for BPA to contaminate groundwater, before it is put into the supply system, has not been fully explored. Contamination may potentially result from disposal of contaminated sludge on land, or from leachate from landfill sites, but this needs investigation.

In utero exposure and potential bio-accumulation

BPA has been found in placental cord blood at the level of micrograms per kilogram (0.4-1.6µg/kg wet tissue),[26] which means that the unborn child is being exposed to this substance. It is imperative to understand the potential exposure of the unborn infant. Accumulation of BPA in maternal blood serum may represent a risk to the foetus. One study, for example, has shown that although most BPA may be initially cleared rapidly, some of the parent compound may remain in the blood, and with repeated exposure, the stable circulating concentration of BPA increases. This study in mice also suggests a substantial individual variation in the BPA levels,

with around 10% of the animals showing approximately 10 fold higher BPA levels than their treatment group averages.[27]

Bottle fed infants are also likely to be exposed due to BPA leaching from the bottle, but in order to determine the full exposure of all infants, studies are needed to determine the levels of this compound in breast and formula milk. A study conducted many years ago in fish certainly suggested that BPA has a low potential for bioaccumulation,[28] but nevertheless, a breast milk study would provide important verification about BPA's ability to bioaccumulate in body fat.

Sensitive sub-populations

Offspring in the womb, babies, and children around puberty are likely to be most at risk from hormone disrupting chemicals. However, in addition, certain animals are genetically more susceptible to the effects of BPA. It has been identified that different strains of rodents have very different sensitivities to oestrogens. For example, BPA stimulated the secretion of the brain hormone that initiates lactation (prolactin) in Fischer 344 rats but not in Sprague-Dawley rats.[29] Similarly, CD-1 mice appear to be 16 times less responsive to oestradiol than some other strains, and this may mean that safety tests conducted with these animals may greatly underestimate disruption of male reproductive development by environmental oestrogenic compounds.[30] It may also be that some humans are more sensitive to BPA and other environmental oestrogens, but this has not yet been fully explored.

Cumulative exposures

In assessing whether effects are likely, for both humans and wildlife, it is important to assess overall total exposure to "oestrogenic" and other hormone disrupting substances. This is because effects may be additive or even more than additive. For example, not only are several pesticides oestrogenic, but also a number of other substances related to BPA have also been found to be oestrogenic in test tube studies. These are shown in Table 2. Bisphenol A, bisphenol F, and bisphenol AF based plastics all represent a potential source of these active compounds. BADGE (Bisphenol A diglycidyl ether) is a starting substance of many epoxy resins used as internal can coatings, but it is also used as an additive, functioning as a stabiliser and plasticiser in blends of PVC and epoxy resins (vinylic organosols), and as a performance enhancer of polyester-based internal can coatings.[31]

Table 2: THE RELATIVE ENDOCRINE DISRUPTING ABILITY OF BISPHENOLS IN TEST TUBE TYPE EXPERIMENTS [32]

Compound	Acronym	Approximate potency relative to potency of oestradiol = 100
17∃-oestradiol	E2	100
Bisphenol A	BPA	0.01
Bisphenol F (Bakelite)	BPF	0.001
Bisphenol A dimethylacrylate	BisDMA	0.001
Bisphenol A bischloroformate	BPACF	0.001
Bisphenol A diglycidyl ether	BADGE	0.0001
Bisphenol A diglycidyl ether diimethacrylate	BisGMA	Perez et al. found no activity in vitro (but active in animals[33,34])

HUMAN EXPOSURE TO BPA FROM FOOD CANS

In 1995, Brotons and colleagues detected BPA in the liquid portion of several types of vegetables (peas, artichokes, green beans, mixed vegetables, corn, and mushrooms) taken from cans with epoxy resin linings.[35] The highest level found would provide an intake of about 80µg of BPA per kilogram of canned food. This value can be seen to be well below the current EU migration limit of 3 milligrams per kilogram of food (mg/kg of food). Similarly, intake levels would be below the current tolerable daily intake (TDI) of 50 micrograms per kilogram body weight per day (µg/kg bw/day) (see below), although it could be argued that these official limits need re-evaluating on the basis of new studies on the effects of BPA at low dosages. With regard to intakes, a 300 gram tin of peas, containing 23µg of BPA, and used to make one portion of pea soup, would result in a dose of around 0.42µg per kilogram of body weight for a 55 kilogram woman.

Industry workers have also evaluated BPA migration from food cans, and found this to range from non detectable in beverages (less than 5µg/kg) to up to 94µg/kg in canned vegetable food, with an average of 37µg/kg.[36]

The Association of Plastics Manufacturers in Europe (APME) have calculated that under worst case conditions, the maximum intake of BPA from food cans is approximately 0.8µg/kg bw/day,[37] although it is likely that most people will have lower intakes.[38] However, it is important to note that all exposure routes need to be considered when trying to establish the worst case daily dose of BPA that might be ingested by, for example, a pregnant woman.

Several Japanese researchers have also investigated the levels of BPA leaching into canned foods. Koji Arizono and co-workers are reported to have found 127ppb (µg/kg) of BPA in canned coffee,[39] which led the manufacturer to reformulate these containers. The next highest level was 10ppb (µg/kg) in tomato juice.[40] Similarly, Kawamura and colleagues have found bisphenol A in some canned drinks, and the highest level found was 40µg in one canned coffee drink. Also, in one of the ten cans of alcohol (sake) they sampled, BPA was detected at 13µg/l.[41]

HUMAN EXPOSURE TO BADGE

Human Exposure to Badge from food cans

In the EU BADGE has also been found in tinned foods. Indeed, in more than 10% of European samples it was found at levels in excess of 1mg/kg of food,[42] the maximum permitted level in the EU (see below). In the light of such contamination, products were withdrawn from sale in Switzerland. Fat-containing foods appear to be particularly affected, including mackerel, pork meat, meat spreads, anchovies, and sardines. In the UK, some sardine samples purchased in 1995 and 1996, and some anchovy samples purchased in 1996, exceeded 1mg BADGE per kilogram of food, but later samples showed some reduction in levels.[43] However, there is some concern that other bisphenolic compounds may have replaced BADGE.

The greatest proportion of BADGE appeared to be in the oil surrounding canned fish. At the time of the UK study, on the basis of food consumption data, it was calculated that human intakes of BADGE from canned food would not exceed 3µg/kg bw/day. [44] However, the following example illustrates that "worst case" intakes could have been higher. The most contaminated sample of anchovies contained 9.3mg/kg, and so a 55kg woman consuming 50 grams might ingest 8.4µg/kg body weight. The presence of chlorohydrin derivatives of BADGE raise particular concern because they have a similar structure as other genotoxic (possible cancer causing) substances[45]. Furthermore, it should be noted that both BPA and BADGE can form adducts on DNA (see[46]). Further tests are being undertaken on the possible cancer causing properties of BADGE . It does not appear to bind to the oestrogen receptor but it does have a small oestrogenic-type effect on cell proliferation in *in-vitro* studies (see[47]).

Human exposure to BADGE from the contact of food with 'susceptors' and drugs with adhesives

Further consumer exposure arises from microwaved foods using susceptors in the packaging. Susceptors are used to achieve local areas of high temperature, in order to brown certain areas of food. In tests carried out in 1992, although most of the susceptors contained no detectable bisphenol derivative, two brands of pizza were packaged with susceptors containing BADGE, and this was found to migrate into the food at a level of 0.1-0.7mg/kg. Manufacturers have been alerted to the problem and subsequent tests indicated that BADGE usage has reduced. There are also other reports of exposure to BADGE arising from the contact of ingested products, for example medicines with adhesives.[48]

BABIES' EXPOSURE TO BPA FROM POLYCARBONATE BOTTLES

In 1993, a study by Krishnan and colleagues at Stanford Medical School found that heating polycarbonate laboratory flasks at 121^{o}C (250 ^{o}F) for 25 minutes released 2-5µg/kg of bisphenol A into water-filled flasks.

Some subsequent studies have not found any measurable amounts of BPA leaching from babies bottles or test discs made of polycarbonate, but other studies in Japan and England have suggested quite significant leaching particularly from older scratched bottles.

Studies which have found no leaching of BPA from babies bottles or polycarbonate test discs, include, for example, one by the Society of the Plastics Industry (with a detection limit of 5µg/kg) and one by the UK Ministry of Agriculture, Fisheries and Food (with a detection limit of 30µg/kg of liquid)[49]. Biles and co-workers also found that when whole polycarbonate bottles were tested with typical fill conditions using normal use conditions no migration was detected, although some migration was noted under exaggerated conditions.

However, some studies in Japan have suggested that more BPA can leach from polycarbonate that has been scratched or is more than four years old. For example, in tests conducted at Nagasaki University in 1998, Koji Arizono and co-workers showed that up to 6.5µg/kg leached from old polycarbonate baby bottles heated up to 95oC for 30 minutes, but new bottles only leached up to 3.5µg/kg (ppb). Arizono also found that scratched bottles from the Philippines leached approximately 30µg/kg (ppb) of BPA and those from Korea leached over 15µg /kg, more than 5 times the amount leached by new bottles.[50]

An unpublished study, commissioned by the UK Government Department of Trade and Industry, conducted at the Laboratory of the Government Chemists in London has also found BPA is released from used babies' bottles, which have been subjected to bottle brushing and/or dishwashing and sterilisation. The amounts leaching into water (representing milk) and 3% acetic acid (representing fruit juice) at elevated and lowered temperatures were recorded. The milk simulant (water) in the used bottles was found to contain 10-20µg/l (micrograms per litre) of BPA, and one value even recorded a level of 50µg/ /l. BPA was not detected in the liquid held in new polycarbonate bottles, which means that if it was present, it was there at levels less than 10µg per litre, which was the limit of detection[51].

Even in the worst case these results can be seen to meet the 3mg/kg EU migration limit (as 50µg/kg is 60 times lower than this). Nevertheless, (even discarding the highest value, which appears an oddity and needs to be checked) it can be concluded that BPA can leach from older scratched babies bottles and that intakes might be around 3µg/kg bw/day for a 2 month old baby. It should be noted that although this is well within the TDI of 50µg/kg bw/day, this is greater than the 2.4µg/kg bw/day effect level identified by vom Saal's work in female mice exposed in the womb. It should also be noted that likely intakes, as calculated below, do not provide a wide margin of safety when compared to the dosages which have been shown to cause effects in other animal studies.

For example, at 2 months of age a human baby consumes approximately160g of milk per kilogram of its body weight per day. If the milk is contaminated at 20µg/l this would mean (assuming 1 litre of milk weighs 1 kilogram) that 1 kilogram of milk contained 20µg, and therefore that 160 grams of milk would yield a dose of 3.2µg per kilogram of the infants' body weight per day (3.2µg/kg bw/day). Similarly, based on the mean consumption figures for breast fed babies, a 3 month old infant ingests around 140µg/kg bw/day, which based on BPA contamination at 20µg/kg yields a dose of 2.8µg/kg bw/day. At 5 months mean milk intake is 70g/kg bw/day, which again, considering the sample contaminated at 20µg/kg, would equate to a BPA dose of 1.4µg/kg bw/day.

The above results give cause for concern. It should be noted that the results use a contamination figure of 20µg/l, which is the amount leaching from used bottles, typically 1 year old, while the intakes that give most concern are those for the very young. This represents the worst case situation, but this level of exposure may occur, as some new born infants may be fed using the feeding bottle previously used by their elder siblings.

It is speculated that following discussions about these results, bottle manufacturers may in future include on the label some advice to consumers, such as a recommendation to change bottles at regular intervals (perhaps 6 monthly). However, a change to safer materials is preferable.

HUMAN EXPOSURE TO BPA FROM POLYCARBONATE TABLE WARE AND WATER AND WINE BOTTLES

Japanese researchers have found increased levels of BPA leaching from old polycarbonate tableware used by children. BPA levels increased to 1.8-7.9ppb (µg/kg) for older products, as

compared to 1-1.9ppb for new tableware.[52] The leaching of BPA into soup was tested by using new and used polycarbonate soup bowls, and filling them with vegetable soup, water, and other liquids at temperatures of 60-95°C, then allowing these liquids to cool for 30 minutes. Both new bowls and old bowls (used for up to 6 years) tested with water at 95°C were found to leach between 0.5-2ppb BPA, whereas at 85 °C, only older bowls leached BPA. It has been reported that Yokohama authorities stopped using one brand of soup bowl that leached particularly high amounts, and that schools are now instructed to discard polycarbonate tableware more than 4 years old.[53]

Kawamura has also looked at leaching from polycarbonate tableware. Ten commercial products contained BPA residues in the polycarbonate at 5-80mg/kg, but migration was only detected in three samples at under 5µg/kg. Four samples with very high levels (rice bowl/mug/soup cup/dish) contained 370-599mg/kg of BPA residues in the plastic. In these products, migration into the different food simulants varied. For example, using n-heptane at 25°C for 60 minutes, migration was between 29-39 µg/kg, whilst for water at 95 °C for 30 minutes, migration was 19-26 µg/kg.[54]

Biles and co-workers investigated leaching from five gallon water carboys. The amount of BPA in water from the five gallon carboys was found to range from ND (not detectable) to 5µg/l (in water stored for 39 weeks).[55] Other scientists have found BPA and other contaminants in mineral water and wine stored in plastic bottles.[56]

HUMAN EXPOSURE TO BISPHENOL COMPOUNDS FROM DENTAL FILLINGS AND SEALANTS

Human exposure to BPA and related oestrogenic substances can also arise from certain composite dental fillings and sealants. Most composites and sealants used in dentistry are based on bisphenol A diglycidylether methacrylate (bis-GMA), and a study of 7 composites and one sealant detected BPA in all the products. The compounds leached included bis-GMA, BADGE, BPA and bis-DMA, which can also be a source of BPA.[57] (For full names see Table 2)

Olea and co-workers considered that although bis-GMA was not oestrogenic in test tube tests, it could be transformed into active compounds in acid or alkaline media, as could BADGE.[58] Mariotti has subsequently identified that bis-GMA is oestrogenic in animals. [59,60]

It should be noted that the amount of BPA reported to be swallowed during the first hour after application of a plastic dental sealant is higher than the 2µg/kg dose of BPA which vom Saal's team suggests can cause effects in mice (see below). For example, Olea and co-workers reported that for one woman up to 930µg was found in the saliva, which is equivalent to a dose of 13µg/kg in a 70kg adult. The average amount for 18 people was around 312µg and even this would result in a dose of 4.5µg/kg in a 70kg adult. This has led some scientists to consider that "women may be placing their fetuses at risk by having dental sealants applied during pregnancy,"[61] although dental sealants (as opposed to fillings) are normally applied to children's teeth rather than the teeth of pregnant women.[62]

The figures on BPA leaching detailed above, and the likely exposure over time have been challenged,[63] and indeed some studies have found no leaching.[64] Nevertheless, whatever the

precise level of exposure to bisphenol compounds from dental practices (and how this varies over time), there are good data to suggest that there is some level of exposure.[65,66] Other workers have found BPA at levels of 4.8-105.6 ppb (µg/kg) in saliva collected at one and three hours after treatment. However, these researchers could not detect any BPA in the blood (at a limit of detection 5ppb) and so concluded that the BPA released from dental sealant may not be absorbed or may be present in the circulation below the limit of detection.[67] They therefore suggested that concern may be unfounded, but this conclusion is premature because only very small amounts may need to be absorbed to cause unwanted effects.

The effects of BPA in rodents, provide a powerful argument for the elimination of all human exposure. It will be very difficult to determine, the clinical relevance of this low-level oestrogenic exposure therefore, it is wise to find safer inert alternatives. Immediate steps must be taken to eliminate the exposure of pregnant women, children and those at the age of puberty.

Official limits

Two types of official limits exist for BPA and certain related compounds.

a) **Migration limits** specify the amount of a chemical that is permitted to migrate into foodstuffs. EU Directives 76/893, 80/590, 82/711, 85/572 and 90/128 summarise the European regulations of polymers in contact with food. Specific migration can be assessed either in foods in contact with polymer material or in food simulants.

For **BPA**, the European Commission Scientific Committee on Food (EC SCF) has established a specific migration limit in food of 3mg/kg (3ppm), *ie* a maximum of 3mg of bisphenol A is permitted to transfer from the plastic into 1kg of food.
The US Food and Drug Administration has not imposed a comparable limit.

For **BADGE**, in 1996 the SCF increased the permitted limit and set a temporary limit for BADGE and its hydrolysis products of 1mg/kg of food or food stimulant. The previous limit was 20µg of BADGE/kg of food, and it was a controversial decision to increase this. In 1999, the 1mg/kg higher limit was re-confirmed, but more toxicity data was requested.[68,69]

b) **Tolerable Daily Intakes** (TDIs) are an estimate of the amount, expressed on a body weight basis, of a contaminant which can be ingested every day over a whole lifetime without appreciable health risk.

TDI FOR BPA IN THE EUROPEAN UNION

In 1986, EC SCF recommended a TDI of 0.05 milligrams (= 50µg) per kilogram body weight per day for bisphenol A in the context of its use in food contact plastics. However, in the light of new data, the SCF planned to re-evaluate BPA in the year 2000.[70]

This European TDI of 50µg/kg bw/day was set before vom Saal's work suggested that BPA can cause effects at low doses (down to 2µg/kg bw/day). However, in August 1997, the UK Committee on Toxicity (COT) re-evaluated the TDI in the light of vom Saal's 1997 study,[71] and other studies (some unpublished) put together by the American Society of Plastics Industry. They concluded that vom Saal's study did not provide any justification to draw any conclusions about the health implications of human exposure, and stated that this study "showed some shortcomings".[72] It could therefore be argued that the COT appears more interested in listening to industry, than in adopting a precautionary approach to protecting human health.

SAFE LEVEL OF BPA IN THE USA

The US Environmental Protection Agency (EPA) has the same "safe" level as the EU. The EPA has established a maximum acceptable or "reference" dose for BPA of 0.05mg/kg bw/day (=50µg/kg bw/day). This was based on a test for cancer.[73]

TDI FOR BADGE IN THE EUROPEAN UNION

In 1999, the SCF determined that no ADI or TDI could be established due to the lack of certain toxicity data.[74]

TDI FOR BPF IN THE EUROPEAN UNION

There is no European TDI for Bisphenol F as it has never formally been requested by industry as a starting substance, monomer or additive for food packaging materials. However, there are concerns that it may now be being used as an alternative to BADGE.[75]

FOOD SAFETY LAW

This briefing concludes that food safety laws both in the EU and the USA need to be amended. As shown above, with regard to BADGE, it could be argued that the EU is not protecting human health if high migration levels are allowed to stand, even in the absence of certain data. Further, permitted intake levels of BPA are above those reported to have subtle effects (see below). Furthermore, little toxicity testing is actually required for certain food contaminants. For example, in the EU, if a substance migrates into food at less then 50µg/kg of food, then no toxicity test, other than to demonstrate absence of genotoxic potential, is required for approving it as a new substance for use in food contact plastics. In the USA, only an acute toxicity test is required. The toxicity testing required for various migration levels are detailed in Table 3 below. Given the concerns about exposure to low levels of hormone disrupting substances, both existing and new substances that migrate into food should be fully tested for toxicity at low dose levels.

TABLE 3: COMPARISON OF TOXICITY TESTING REQUIREMENTS IN THE EU AND USA

Level of exposure (as level in food)	*EU requirements*	*USA requirements*
EU: None detectable USA: 0.5 µg/kg	3 mutagenicity tests	None
EU: *l.o.d.- 0.05mg/kg USA: 0.5µg/kg - 0.05 mg/kg	3 mutagenicity tests	Acute toxicity test
EU: ≥0.05 - 5 mg/kg USA: 0.05 - 1 mg/kg	3 mutagenicity tests 90-day oral study Bioaccumulation	Two 90-day oral studies (1 in utero exposure rodent and 1 non-rodent)
EU: >5 mg/kg USA: >1 mg/kg	ADME** Reproduction Teratogenicity Lifetime toxicity/ carcinogenicity	Lifetime toxicity/ carcinogenicity studies Others to be decided

* l.o.d. = limit of detection

** ADME = absorption, distribution, metabolism, excretion

Potential effects in humans: Mammalian studies

In toxicology, the possible effects on humans are generally deduced from studies on rats and mice. It has been known for many years that bisphenol compounds are able to mimic the female hormone, oestrogen,[76,77] but it was not until the late 1990s that researchers began to worry that this may lead to effects at relatively low levels of exposure. Much of the concern is focussed on the potential effects on the unborn child, because the sex hormones play a crucial role during foetal development. Babies may also be at risk. For example, during the first three months of life, male babies have high levels of male hormones (around 50% of adult levels).[78,79] It is not known exactly why this is, but it is believed that the subsequent behaviour of the individual is imprinted at this time.[80] Therefore, interference in the hormonal processes of a baby may have significant consequences to development.

A number of studies into the effects of BPA exposure are outlined below, and several are contradictory. Industry studies have not replicated those of other workers, and have put forward several explanations for this, including the natural variability within the rodent litter, which could give rise to false positive or false negative results if just one offspring per litter is examined. For example, from the table, it can be seen that both vom Saal's team, and Gupta and co-workers, only examined one male mouse per litter, whereas the industry studies looked at up to 3 or 4 male offspring per litter.

Differences in the natural variability within the litter can not explain many of the effects that have been noted in other studies. For example, several studies have now been conducted, on both on young male and female offspring and these suggest that effects can occur at dose levels of 100µg/kg bw/day and below. One such study undertaken by vom Saal's team looked at females exposed in the womb, and took full account of the position in the womb and the natural variability within the litter. It suggested that a dose level of just 2.4µg/kg bw/day given to the pregnant mother on days 11-17 of gestation may affect the time of onset of puberty. Therefore, several studies now indicate there may be little, or no, margin of safety with regard to current human intakes.

The issue of low dose may be to some extent authoritatively resolved later in 2000 when the National Toxicology Program in the USA holds a meeting to review the effects of "doses below the currently accepted No Observed Adverse Effect Level for that substance". The outcome of this inquiry will affect testing strategies in the USA, and will doubtless be an important input into the risk assessment of BPA in the EU and elsewhere.

Table 4 identifies the dose levels that various research teams have shown can cause effects on male rodent offspring, exposed in the womb either via the feed or via gavage, of their mothers. Table 5 illustrates some of the studies that have been undertaken into the effects of BPA on the male offspring of rats exposed to BPA via the drinking water of their mothers. These are described in greater detail in the text, as are other studies that suggest effects at low dose levels. This briefing does not summarise the numerous studies that confirm BPA acts as an oestrogen mimic.

Table 4: Effects in rodents exposed in utero – exposed via feed or gavage of dams

Researchers	Days of Pregnancy females dosed	Age male offspring examined	Dose given to mother during pregnancy – via feed or gavage µg/kg bw/day					
			0.2	2	20	50	100	200
Vom Saal et al. [81] (by mouth by pipette)	Days 11-17 CF1 mice 5-7 litters 1male (m) per litter	180 days		Yes +pw **-ew** -sv **+pg** **-bw**	Yes **+pw** -se -ew^{ns} +pg^{ns}			
Gupta et al. [82] (fed in corn oil)	Days16-18 CD1 mice 15 litters 1 male/litter at each time	3 days 21 days 60 days				Yes +pw +pw **+pw/**-ew		
Ashby et al[83] (repeat of vom Saal) (by mouth by pipette)	Days 11-17 CF1 mice 8 female/dose 3m per litter	180 days		Some effects pw tw But believed due to **+bw**	Some effects pw tw ew -believed due to +bw^{ns}			
SPI (Cagen et al.) [84,85]	Days 11-17 CF1 mice 28 females per dose. Maxm of 4 m/litter	90 days	No	No	No pw/pg/ sv/ew			No
Fialkowski, Talsness & Chahoud [86] (by gavage) (NB. Some effects noted were more pronounced at 100ug dose than at 50,000 ug)	Days 6-21 Rats	70 days 170 days					Yes At 70 and 170 days -tw -ew +t +sn	

Notes to Tables 4 and 5

+pw or -pw	increased or decreased prostate weight.
p	prostate
ew	epididymal weight
sv	seminal vesicles
pg	preputial gland
se	sperm efficiency (daily sperm production per gram of testis)
bw	body weight
tw	testicular weights
sn	sperm number
t	testosterone level
pw or pw	figures in bold represent the greatest effect.
ns	effects were noted but these were not statistically significant
*	effects on ventral prostate wt - considered due to intra-litter variability
	Actualdose levels were 0, 0.005, 0.05, 5 and 50mg BPA/litre
	Corresponding to daily intake ranging from 0.001 to 10 mg/kg/day

Table 5: Effects in male rat offspring – exposed via dam's drinking water

Study	BPA administered to rats in mother's drinking water at ppm or mg/litre dose								
	0	0.005	0.01	0.05	0.1	1	5	10	50
Sharpe et al.[87] Dosed females at 10 weeks old, 2 weeks pre-mating, 2 weeks mating + 21-22 days of pregnancy and 22 days lactation.						Yes -ts -sp			
Dimond et al.[88] Dosed as above	No		No		No	No		No	
Elswick et al.[89] (CIIT) Dosed from day 2 of pregnancy to 21 days old	No	No		Yes +pw* see notes to table 1			Yes +pw* see notes to table 1		Yes +pw * see notes to table 1

EFFECTS OF IN-UTERO EXPOSURE ON MALE AND FEMALE OFFSPRING

Fred vom Saal and Wade Welshons and co-workers showed that male mice exposed to low levels of BPA in the womb (on gestation day 11-17) exhibited; increased prostate weight, reduced sperm production, reduced size of seminal vesicles, [2] and increased size of preputial glands.[3]

A statistically significant increase in prostate weight of 30% was observed at a BPA dose of 2µg/kg, and a 35% increase was similarly noted in the male offspring of mothers dosed with 20µg/kg.[90] Also, at 20µg/kg BPA, a statistically significant decrease (20%) in daily sperm production per gram of testis was observed. However, epididymal[4] weights were significantly reduced in the group exposed to 2µg/kg in the womb, whilst this reduction was not so apparent in the higher dosed group. Similarly, seminal vesicles tended to be smaller in the group exposed to 2µg/kg in the womb as compared to those of the controls, but in the mice exposed to 20µg/kg they were not significantly different from the controls. Also, males exposed to the 2µg/kg dose of BPA had significantly larger (35%) preputial glands, while in those mice exposed at 20µg/kg this trend was not statistically significant.[91] Vom Saal's studies also found a significant reduction in body weight in the mice exposed to 2µg/kg of BPA, but no significant effect on body weight in the higher dose group. Both testis and epidydymal weights were significantly correlated with body weight.

These experiments led to world-wide discussion about the phenomenon of inverted U shaped dose response curves, where low dose levels were suggested as being potentially more harmful than higher doses.

In 1999, the vom Saal team also published details of the effects on female offspring of mice exposed in the womb on days 11 to 17 of gestation. The BPA dose was 2.4µg per kg body weight, which is a similar dose to that which caused effects on the male offspring, and a level equivalent to that typically found in the environment.[92] The study suggested that trans-placental exposure to low doses of BPA could bring on early puberty in female pups. At postnatal day 22, female offspring surrounded in the womb on one or both sides by other females, were significantly heavier than control females from the same in-utero position but not treated with BPA, particularly the females that had been between two other females in the womb. The workers concluded that prenatal exposure to a dose of BPA, comparable to levels found in the environment, altered postnatal growth rate and reproductive function in female mice. However, individual differences in endogenous (self-made) oestradiol influenced the effects of this exposure. Female mice offspring surrounded by two females in the womb have higher endogenous oestradiol levels and these were the ones where BPA exposure reduced the number of days between vaginal opening and first vaginal oestrus ovulation which is highly correlated with first post-pubertal ovulation. This suggests that animals and humans with higher endogenous oestrogen levels may be particularly sensitive to "oestrogenic" pollutants.

Dr Chhanda Gupta and colleagues of the University of Pittsburgh have also shown that in-utero exposure to a low dose of BPA can affect prostate size in male mice.[93]

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[2] Fluid from the seminal vesicles makes up the bulk of the ejaculate volume in mice.

[3] The preputial glands are involved in social communication in territorial marking.

[4] The epididymides play a role in sperm maturation and storage.

This work showed that 50µg/kg/day of BPA given to pregnant mice on days 16-18 of gestation induced the following in the male offspring; – enlarged ano-genital distance, decreased epididymal weight, and increased weight of prostate, which were also shown to have increased androgen receptor binding activity. The enlargement of the prostate was seen in animals of 3, 21 and 60 days old, but the difference was greatest in the 60 day animals. No effect was noted on weight of the testes or vas deferens. The dose given was chosen because 50µg/kg/day is the level considered safe by the US Food and Drug Administration. These workers also found that the synthetic oestrogen, DES (diethyl stilboestrol) had different effects on prostate weight at high (200µg/kg bw) and low (0.1ug) doses, such that the higher dose caused a decrease in weight whilst the lower dose caused an increase.

Chahoud and Talsness and co-workers at the Freie Universitaet in Berlin have also looked at the effect of exposing pregnant rats to BPA at dose levels of 100 and 50,000 µg/kg bw/day on day 6 through to day 21 of pregnancy. Effects were noted in the offspring of the rats dosed at levels down to 100µg/kg bw/day, and so a NOEL "no observed effect level" was not established. In the females, effects on the reproductive cycle were noted, and vaginal opening was delayed in the low dose group and accelerated in the high dose group.[94]

In males, examination of the offspring after birth at different ages revealed effects which were different at the high and low dose levels, and sometimes more pronounced in the lower dose group.[95] For example, at postnatal day 70, absolute testes weights were significantly decreased with the lower dose, but slightly (not significantly) increased with the 50,000µg dose level. Also, both BPA dosages gave rise to significant delays in preputial separation, but this effect was more pronounced in the low dose group. In addition, the sperm number was significantly increased by both the high and low doses of BPA at 170 days of age, but at 70 days of age, the sperm number in the low dose group (100µg/kg bw/day) was significantly increased, whereas in the high dose group (50,000µg/kg bw/day), it was only slightly increased and non-significant.

Cagen and colleagues working on behalf of the Society of the Plastics Industry (SPI) have published a study conducted at MTI Research laboratories, which tried to replicate vom Saal's findings in male mice. However, they failed to do so, and concluded that vom Saal's work was invalid.[96,97] Like vom Saal, these industry-funded workers exposed pregnant rats to BPA on days 11-17 of gestation at dose levels of 2 and 20µg/kg bw/day, but they also looked at doses of 0.2 and 200µg/kg bw/day. Groups of 28 female mice were used and a maximum of 4 males per litter were retained until 90 days (13 weeks) old. The positive control was 0.2µg/kg bw/day of diethylstilboestrol (DES). At 13 weeks of age the male pups were examined. At all dose levels, they found no effects of BPA on prostate, preputial gland, seminal vesicle or epididymis weights, and no statistically significant effects on daily sperm production.

However, in this experiment it appears that there were no effects due to DES administration at 0.2µg/kg. The fact that this positive control did not "work", has led to the counter suggestion that this work is invalid, and not that of vom Saal's team. It has been suggested that the industry mice may have been handled such that they were under stress, and hence the controls were already off the baseline, thus masking any effects of the low dosages. DES can give rise to oestrogenic responses at a lower dose, including the turning on of specific oestrogen related genes, but tissue weights are not particularly sensitive to the hormonal activity of a compound.

Nevertheless, in any experiment the positive controls must be positive.[98] Other possible explanations for not being able to confirm the findings of vom Saal have been suggested, including the difference in age the offspring were examined, and the natural intra-litter variability, which is discussed in more detail below (see under Elswick and co-workers).

John Ashby and colleagues have published reports concluding that BPA causes no effects at low dose levels. In an experiment designed to replicate the work of vom Saal (but using larger sample sizes), the workers suggested that BPA at 2µg/kg/bw/day and 20µg/kg bw/day (given to pregnant CF-1 mice by micro-pipette on days 11-17) produced no effects on prostate weight or sperm efficiency in the male offspring. [99] However, effects on body weight were noted, and adjusting for body weights became an issue for prostate, testes and epididymal weights. Thus, they concluded that "the slightly elevated prostate weights observed in the bisphenol groups are likely due to the increased body weight in these same groups." The marginal increase in testicular and epididymal weights was considered to be an equivocal finding because the testis/body weight and epididymal/body weight ratios were essentially identical in the BPA and control groups. This conclusion was reached despite the fact that the elevated testis weights in the BPA groups were significant even after adjustment for body weight. Similarly the marginal increase in daily sperm production, but not in sperm efficiency, for the BPA groups was considered to be of low biological significance, and hence an equivocal finding.

The interpretation of this experiment is interesting, as it could be argued that some effects due to BPA administration do seem apparent. However, Ashby and co-workers also concluded that as significant dam effects were seen for several parameters, the statistical unit used in such experiments should be the litter as opposed to the individual.

Ashby also identified several other possible reasons for non-replication of the vom Saal study including: lack of a radio to soothe the animals; phytoestrogens in the diet; body weight differences, including isolated and group-housed effects; and genetic differences. Like Cagen et al (1999) (see SPI study above), Ashby's team also failed to find any effects on prostate weights due to DES at 0.2µg/kg bw/day. Certainly, in any good experiment, the positive controls should show a response, but as outlined above, it is possible that prostate weight is not a sensitive enough parameter to show an oestrogenic effect with this dose level of DES.

Ashby and colleagues also looked at the female mice offspring and concluded these animals underwent normal sexual maturation and showed no differences in the mean day of vaginal opening and no significant differences in reproductive tissue weights at termination.[100]

Studies on rats exposed via drinking water

Richard Sharpe of the UK, at a conference in San Francisco in 1996,[101] reported reduced testes size and sperm production in the male offspring of pregnant rats given BPA at 1ppm (mg/l) drinking water.[102] This approximates to a dose of 125-370µg/kg bw/day (dependent on age and amount drunk). However, industry workers could not replicate similar effects (see below), and Sharpe and co-workers subsequently noted unexplainable effects in testes weight in some of their control animals.[103]

Dimond and colleagues, working on behalf of the chemical industry, tried to replicate Sharpe's study. They used the same treatment periods and dose levels as Sharpe, but found no effects on reproductive parameters of male offspring of Han Wistar albino rats. The reproductive

parameters looked at included testes, prostate and preputial gland weight, sperm count, daily sperm production and testes histopathology. Dose levels were 0, 0.01, 0.1, 1.0, or 10 mg/l in drinking water, and dosage of the females began at 10 weeks of age, and continued throughout a 2-week pre-mating period, 2 weeks of mating, 21-22 days of pregnancy, and 22 days of lactation.[104] (Combining water consumption values in females during premating, gestation and lactation periods, the dose levels in water were considered to correspond to approximately 0, 0.002, 0.02, 0.2 and 1.8 mg/kg/day respectively)

Elswick and coworkers working at the Chemical Industry Institute of Toxicology (CIIT) conducted a similar study with BPA in drinking water and failed to find any impairment of reproductive function in male Sprague Dawley rat offspring exposed to low doses of BPA in the womb, as these male rats were carried through to mating.**[105]** Two replicate blocks of 8 female rats were given BPA in the drinking water at dose levels of 0, 0.005, 0.05, 5 or 50 mg/l from the second day of pregnancy to 21 days after birth. The resulting daily intakes ranged from 0.001 to 10mg/kg bw/day, and depend on the amount drunk and the weight of the rat. No effect was found on ano-genital distance or preputial separation. Similarly, analysis for androgen receptor in ventral prostates from the 41 day old rats did not show any BPA related changes, and at 177 days old, the BPA exposed rats did not display significant differences in hormone levels, sperm counts or ventral prostate androgen receptor levels.

However, these workers did find a significant increase in the weight of the ventral prostates in the 0.05,5 and 50 mg BPA/litre dosing groups when the 177 days old rats were examined, although this was not dose related and there were no histological changes. These workers investigated 1 or 2 males chosen randomly from the litter, and suggested that the large intra-litter variability of the ventral prostate weights may be a confounding factor in these results. They concluded that apart from the equivocal effect on ventral prostate weights, the data indicate that under the indirect pre- and postnatal BPA exposure conditions (drinking water of the dam and lactation) the differentiation and function of the reproductive system in male rats appeared to be unimpaired. However, it could be argued that it was selective to dismiss the effects on prostate weight, largely because these were not dose related, *ie* they did not fit the standard linear dose response curve. However, a further publication from CIIT suggested that because of the natural intra-litter variability, false negatives or false positive results regarding effects on prostate weight, may be generated more than 50% of the time, if only one pup per litter is randomly selected.[106]

Female offspring up to 10 months of age, and from the same dosing regime have been examined. It was concluded that in female offspring, exposed in the womb and via the lactation of their mother, the differentiation and function of the reproductive system appeared to be unimpaired.[107]

Markey and co-workers have shown effects on female offspring exposed in the womb to low doses of BPA.[108] These workers exposed pregnant CD-1 mice to 25-250 µg/kg BPA from day 8 to day 20 of gestation, and assessed the female offspring for effects on the oestrus cycle and histology of the vagina. In both dose groups they found persistent oestrus when assessing oestrus cyclicity over a 14 day period. This work is important because it shows in-utero exposure to low, environmentally relevant doses of BPA induces marked changes in the vaginal cells and changes in the reproductive cycle.

EFFECTS ON YOUNG MALES

Workers in Japan have found that effects can also be seen if male mice are directly dosed with low levels of BPA.

Ohsako and colleagues[109] reported that BPA has a direct effect on sperm production in 13 week old mice given a daily dose for 6 days (intubated). The lowest observable effect level (LOEL) was estimated to be 20µg per kilogram body weight.

Takao and co-workers[110] have also reported effects on 5 week old male mice (strain C57BL/6) given BPA for 4 or 8 weeks at a dose of 0.5µg/ml or 50µg/ml in drinking water. The dose ingested was not calculated but assuming a male mouse drinks 5ml of water a day and weighs 30g, a dose of 0.5µg/ml is approximately 83µg/kg bw/day. As puberty in mice is reached around 6-7 weeks of age, this represents pre-pubertal and young adult exposure.

Both doses of BPA over 4 and 8 weeks tended to decrease plasma free testosterone levels dose-dependently, although the decrease was only statistically significant in the 50µg/ml dose given for 8 weeks. Changes in the morphology (multi-nucleated cells) of the seminiferous tubules in the testis were noted in some of the samples from both the high and low dose animals exposed for 8 weeks, but there were no abnormal changes seen in any of the groups exposed just for 4 weeks. They concluded that exposure to BPA around puberty may directly disrupt the male reproductive tract.

EFFECTS ON FEMALES

Colerangle and Roy found that 100µg/kg/day of BPA affected the mammary gland in female Noble rats, and the sort of effects caused by BPA might be a risk factor for the development of cancer.[111] However, it seems that the study may not have been conducted to best practices,[112] but nevertheless the effects noted by Colerangle and Roy are important. Furthermore, it is perhaps rather chilling to note that with DES (diethylstilboestrol), a more potent but structurally similar compound, an increased susceptibility to cancer is evident not just in the offspring exposed in the womb, but also in the offspring of these offspring.[113]

Ben-Jonathan and Steinmetz have shown that BPA can increase prolactin release and have highlighted that it also stimulates uterine, vaginal and mammary growth and differentiation.[114] Even at low doses, given by a continuous release capsule that supplied a dose of around 300µg/kg per day, these workers have reported that BPA caused cell changes in Fischer 344 rat uterus and vagina, and the alterations caused were almost identical with those produced by oestradiol.[115] This experiment also identified that the vagina appears to be particularly sensitive to the oestrogenic actions of BPA.

EFFECTS ON MAMMALIAN BRAIN DEVELOPMENT AND BEHAVIOUR

Christian and Gillies from Imperial College Medical school in London have also found that bisphenol A can affect brain cells in test tube type experiments. They therefore suggest that, if

these substances reached the developing brain, they might be able to alter fertility and sexual behaviour. Maternal oestrogen apparently does not enter the foetal brain, but BPA has a different structure and so may behave differently.[116]

Taisen Iguchi has shown that BPA can indeed very quickly reach the foetal brain, as he has injected BPA into the blood stream of a pregnant mouse on day 17 of gestation and found it in the brain of the offspring just 30 minutes later.[117]

Farobollini and co-workers have shown that exposure to an environmentally relevant dose of BPA can affect brain development in offspring.[118] They exposed female Sprague Dawley rats to 40µg/kg bw BPA from 10 days before mating until the weaning of the pups. The behaviour of the male and female offspring, exposed both in the womb and via lactation, was affected differently. However, contrary to what had been expected, masculinization of the females was not observed. In males both the motivation to explore and anxiety were reduced, while in females, the motivation to explore, and motor activity were reduced. This experiment confirms that exposure to a weak environmental oestrogen in the period of sexual differentiation of the brain can influence adult behaviour.

Aou from Japan has also studied the effects of BPA on behaviour and it has been reported that his findings suggest that exposure to low doses of BPA causes the behaviour of females and males to become similar, so that the normal differences are no longer present.[119] However, as of early 2000 the details of this study were not publicly available.

WWF's Conclusions and Recommendations for BPA

Main conclusions

1. Human and wildlife exposure to BPA and related "oestrogenic" compounds should be eliminated.

2. Current human and wildlife exposures to bisphenol A are such that effects can not be ruled out.

Policy recommendations

3. Wildlife exposure to bisphenol A should be eliminated, and therefore releases to the environment should be prevented. This is because even relatively low levels can cause effects, and furthermore, there is the potential for interaction with other endocrine disrupting substances, which are already found as contaminants in biota and in the environment.

4. In the interim to ensuring that EU legislation is in place to eliminate BPA exposure, governments should provide information to mothers on the hazards associated with BPA, and the available alternatives to polycarbonate feeding bottles for babies. In order to allow for informed public choice, consumer articles including babies' feeding bottles and tableware should be labelled indicating their constituents. Furthermore, governments should advise parents to discard polycarbonate bottles and tableware when they appear worn.

5. The EU should publish new information as soon as possible. In particular, the UK Department of Trade and Industry sponsored study, undertaken at the Laboratory of the Government Chemists, that identified BPA releases from babies'feeding bottles, should be published immediately.

6. There is a need to review the Tolerable Daily Intake (TDI) of 0.05mg/kg bw/day for BPA, which was recommended by the European Commission's Scientific Committee for food (SCF) in 1986, in the context of its uses in food contact plastics. There is also a need to reduce the legal limit of BADGE in foods, until further data are provided to verify its safety beyond reasonable doubt.

7. Food safety laws in the EU and USA need to be amended. In particular, both existing and new substances that migrate into food should be adequately tested for toxicity at low dose levels.

8. Human exposure to bisphenol A and related oestrogenic compounds should be eliminated. Therefore, the use of these substances in food packaging materials, and in the manufacture of substances liable to come into contact with food and drink, should be phased out.

9. Companies should be given a duty to report all releases of BPA, and hence this compound should feature on the European Pollutant Emission Register (EPER).

Research needs

10. Further research is needed to determine all the uses of BPA that are liable to result in human exposure

11. Studies are particularly needed to determine the current in-utero exposures of human babies, and the exposures of infants and pre-pubertal adolescents. However, such research should not delay controls on known exposure routes.

12. Further studies should be undertaken to clarify the effects of low doses of bisphenol A and related compounds, including potential behavioural effects.

13. The hormone disrupting ability of compounds related to bisphenol A need to be investigated fully, as does the potential human and wildlife exposure to these substances.

14. Research should be undertaken to examine the level of BPA in terrestrial and aquatic ecosystems, including the levels in sediments. More research is also needed on the effects of BPA on a number of species, including sediment dwelling organisms.

15. Breast milk samples should be analysed for bisphenol A and related compounds. It is regrettable that a UK pilot study which was to have included monitoring of BPA in breast milk, will now not include BPA, perhaps due to a lack of resources.

16. The effects of BPA on exposed workers should be fully investigated.

17. There is a need to find safer alternatives to BPA, including alternative ways that do not rely on the use of chemicals at all. For example, as well as finding alternative can coatings to those which contain bisphenolic substances, it would be beneficial to find ways to encourage a greater reliance on fresh locally grown produce.

18. There is a need to ensure that independent scientists are properly resourced.

19. There is a need to involve stakeholders in determining what research is undertaken.

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