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Polychlorinated Biphenyls: The Occurrence of the Main Congeners in Follicular and Sperm Fluids

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Summary: We have studied the presence of polychlorinated biphenyls in human body fluids associated with reproduction. Since the polychlorinated biphenyls represent a family of compounds, 3 of the main congeners of this family were selected for this study. The distribution of these 3 congeners was investigated in 37 specimens of follicular fluid and in 16 specimens of sperm fluid. Both fluids showed a similar, low contamination with total polychlorinated biphenyls (ca. 10 μg/kg on average), but it was evident that the follicular fluids preferentially accumulated the more highly chlorinated components. This finding must be taken into account when interpreting the concentration levels of the main congeners in relation to total pollution and the toxic potential of polychlorinated biphenyls.

Introduction

The polychlorinated biphenyls make up a group of non-polar organochlorine compounds consisting of more than 200 individual components (the congeners) and these are a ubiquitous factor in the environment. They are present in the air, the ground water, and the soil, and they are found in and on plants. Due to their chemical stability, they accumulate in the adipose tissue of animals and, in the final link in the food chain, they find their way into man (1–7).

Among the chlorinated hydrocarbons, the polychlorinated biphenyls require special attention, and they must be considered separately from insecticides like dichloro-diphenyl-trichlorethane (DDT) and hexachloro cyclohexane (1). As commercial industrial products, polychlorinated biphenyls have long been used as lubricants, hydraulic fluids, softeners, insulating fluids or flame retardants (2). Polychlorinated biphenyl formation and emission have also been established for many different technical processes. Incineration temperatures that are too low (below 1200 °C) lead to the appearance of polychlorinated biphenyls in the flue-ash of the garbage-incineration facility, and additional polychlorinated biphenyls are formed from the incineration of other organo-chlorine components, such as chlorinated benzenes. At the same time, polychlorinated biphenyls can escape into the ground-water of garbage disposal sites (1, 2, 8).

When deployed in the technosphere, they continue to reach the outside world and persist there.

Since their introduction in 1929, 1 million tons of polychlorinated biphenyls have been manufactured and partly introduced into the environment. Since their toxic effects on nature, both organic and inorganic, became known, their use has been increasingly curbed (2). Since February 1978 the Federal Republic of Germany has forbidden their use expected in closed systems.

Yet these substances, because they are difficult to degrade, continue to show up in the human organism. Lipophilic substances like organochlorinated hydrocarbons can be taken up through the gastro-intestinal tract, cutaneously or through the surface of the lung. Today, polychlorinated biphenyls enter the environment, and then the food chain, firstly by escaping from "closed systems" like small condensors, transformers or hydraulic systems, and secondly by accidental loss from open systems, e. g. during waste-oil incineration (1, 2, 8).

In the organism polychlorinated biphenyls are converted by cytochrome-P-450-dependent monooxygenases into phenols, then conjugated with glucuronic acid. This biotic transformation of the individual congeners varies, however, depending on the number and structure of the chlorine atoms (1, 8). In addition other foreign substances, as well as endogenous compounds of the organism, can influence the metabolism of the polychlorinated biphenyls, by inducing or inhibiting the cytochrome-dependent monooxygenases; examples of such compounds are barbituric acid, phenylbutazolidines, sex hormones and technical substances like DDT (1, 2, 8).

The occurrence of polychlorinated biphenyls in the human organism has been associated with numerous disorders. Polychlorinated biphenyls are suspected of influencing reproductive functions and, because cancer- and teratogenesis cannot be ruled out as possibilities, they represent a danger for population groups which are particularly exposed to pollution (2, 9–15). Chronic feeding tests with different bird species produce alterations of the reproduction rate with declining egg production and increasing teratogeneous damage, such as skeletal anomalies and eye malformations. A rise in the rate of spontaneous abortion and premature delivery is related to a high polychlorinated biphenyl exposure. The local influence of polychlorinated biphenyls on oestrogen receptors and interference in prostaglandin biosynthesis may be responsible for the influence on the reproductive functions (1, 2, 13, 16).

Because they are lipid soluble, polychlorinated biphenyls can pass through the placenta. Toxic effects in embryos have been found in various animal species (1, 2, 4). Also in man, a correlation between prenatal exposure and altered birth-weight has been reported (17).

Since 1970, studies have been carried out on the polychlorinated biphenyl levels in different human body fluids and tissues. These determinations are generally reported in total polychlorinated biphenyl content, which is unsatisfactory, because different congeners have different biological effects (18, 19).

The different congeners of the polychlorinated biphenyls display important difference in toxicity, which can be estimated by the enzyme induction rate. In the guinea pig, the LD 50 of strong inducers of the cytochrome-P450-dependent monooxygenases is 100 times less than that of weak inducers. A single dose of 15 µmol/kg of polychlorinated biphenyl No. 169 leads to atrophy of the thymus and hepatomegaly with single cell necrosis. However, to achieve the same response from a single dose of a weak monooxygenase activator, such as polychlorinated biphenyl No. 118, it is necessary to administer 1120 µmol/kg (1, 2).

Reliable analytical methods are now available for the separation, identification and quantitative determination of individual congeners. These include capillary-gas chromatography with temperature programmable vaporisers, and high sensitivity detection by electron capture. In the present paper the distribution of the 3 congeners, No. 138, 153 and 180 according to *Ballschmitter* (see tab. 1), have been determined for follicular and sperm fluids, and compared with the known distribution pattern in human milk and commercial products.

Tab. 1. Main congeners of polychlorinated biphenyls, according to *Ballschmitter*.

No. 138	2,2′,3,4,4′,5′-Hexachlorobiphenyl
No. 153	2,2′,4,4′,5,5′-Hexachlorobiphenyl
No. 180	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl

Materials and Methods

The follicular and sperm fluid samples used in this investigation were from the in vitro fertilization programme of the University Clinic for Gynecology and Obstetrics in Bonn. The follicular fluid (37 samples) was from patients suffering from sterility of organic origin.

The follicular aspirates were acquired after laparoscopic or transvaginal puncture under ultrasonographic control, after a stimulatory treatment with clomiphene, human chorionic gonadotropin or human menopausal gonadotropin/human chorionic gonadotropin. The puncture was performed 36 hours after an intramuscular injection of 10000 IE human chorionic gonadotropin (20). After separation of the pre-ovulatory follicle cells, the surrounding fluid was stored at −20 °C till analysed.

Sixteen sperm samples were acquired by masturbation, frozen and stored until analysis. The donors were healthy men with normal spermiograms. The criteria for the diagnosis of spermal normality are a sperm count of $> 20 \cdot 10^9$/l, of which more than 40% are motile and more than 40% show no exceptional morphology.

The samples used in this study were prepared according to a modified method proposed by the Senate Commission of the DFG (German Research Society) for the residue analysis of insecticides, followed by gas chromatographic separation (21, 22). We used the following modifications, especially for the gas chromatographic injection and separation procedure.

The preliminary separation of the samples on a column filled with Florisil (eluate of 1000 µl) was followed by a capillary gas chromatographic separation with programmed temperature injection system (PTV) and electron-capture detection (23, 24). We used a Perkin Elmer Sigma 2000 gas chromatograph with the LCI-100 PE integrator and a 50 m × 0.32 mm DMS Permaphase quartz capillary column with 1 µm coating (Perkin Elmer, Bodenseewerk, Überlingen). The analytical results were confirmed by a second chromatographic run with a 30 m × 0.25 mm SBP-608 fused silica capillary column, film thickness 0.25 µm (Supelco, Inc., Fa. Amchro, Sulzbach/Taunus).

A volume of 1 µl was injected using a temperature programmable vaporiser. The separation was temperature-programmed: starting temperature 65 °C, hold for 3 minutes; then heating to 150 °C in 10 °C steps within 8 minutes, hold for another 2 minutes; then heating to 230 °C at 2 °C /minute and hold it for at last 20 minutes. The linear gas velocity was set at 20 cm/s.

A further 10 chlorinated hydrocarbons: hexachlorocyclohexane isomers, hexachlorobenzene, heptachloroepoxide, dieldrin and DDT and its degraded products were also determined. The concentrations of the polychlorinated biphenyl congeners No. 138, 153, 180 (according to *Ballschmitter*, see fig. 1) were determined by using an external standard of high purity (> 99%) obtained from Dr. *Ehrenstorfer*[1]) (8, 18, 19). Recoveries from samples with pesticidal compounds at levels of 10 to 1000 µg/kg were consistently in the range of 80–100%. The electron-capture detector was shown to give a linear response for concentrations between 6 and 100 µg/kg. The variation coefficient was 3.4% of the mean for the detection of 2,2',3,4,4',5'-hexachlorobiphenyl (No. 138) at a concentration of 25 µg/kg.

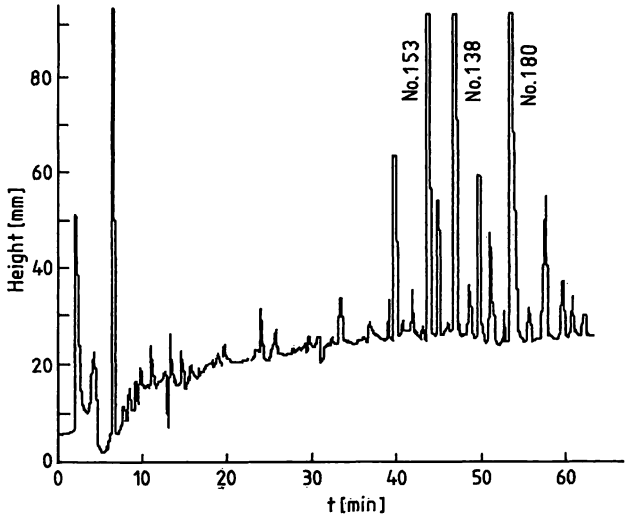

Fig. 1. Separation of 100 µg/kg Clophen A 60 (commercial polychlorinated biphenyl mixture) on a DMS Permaphase column 50 m, 1 µm coating, Perkin-Elmer capillary gas chromatograph series 2000, sample volume 1 µl.

Results

We determined the concentrations of 3 of the main congeners of the polychlorinated biphenyls (138, 153, 180) in follicular and sperm fluids and compared them with each other. The total concentration of the 3 congeners lay between 0.5 and 24.2 µg/kg for the follicular fluid (mean value 5.8 µg/kg) and between 1.8 and 58.6 µg/kg (mean value 11.7 µg/kg) for the sperm fluid (see fig. 2).

The concentrations of individual congeners show a much more differentiated picture, with strikingly different patterns for follicular and sperm fluid. While congener No. 180 (see tab. 1), appears to accumulate most in the follicular aspirate, it is congener No. 153

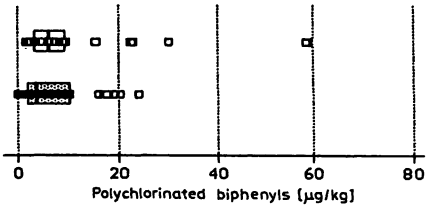

Fig. 2. Differences in the concentrations of polychlorinated biphenyls No. 138 (2,2',3,4,4',5'-hexachlorobiphenyl), No. 153 (2,2',4,4',5,5'-hexachlorobiphenyl) and No. 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl) in the sperm fluid from 16 normal sperm men (□) and in 37 follicular fluid samples (▨); the figure shows the 16–84 quantil of distribution (68% range); the respective bars indicate mean distribution and boxes the individual values.

that shows the highest concentration in the sperm fluid, followed by No. 138 and then No. 180. This means that follicular and sperm fluids show opposite patterns of accumulation with respect to the degree of chlorination of the polychlorinated biphenyls (see fig. 3–5).

In order to compare the concentration levels found in the follicular and sperm fluids with the pattern of a commercial polychlorinated biphenyl mixture (Clophen A 60) and the levels found in an extract of human milk, we took the sum total for the concentrations of all 3 congeners as 100% and presented the percentile distribution in a graph (see fig. 5).

Discussion

The samples we studied showed varying degrees of contamination with polychlorinated biphenyls; the concentrations found were in accordance with the results of other investigations (5–7, 15, 25). In the present work, however, we were concerned with the differential accumulation of individual polychlorinated biphenyls, which differ only with respect to the number and positions of the chlorine substituents. Superficially, this group of polychlorinated biphenyls appears to be homogeneous, yet the individual congeners in part differ considerably with respect to their persistence in the environment and their toxicity (1).

Chlorinated biphenyls with a coplanar structure seem to be especially toxic, since they are able to induce the metabolizing monooxygenases of the cytochrome P-450 and P-448 type. They bind reversibly to a cytosolic receptor complex (Ah-receptor for "aromatic hydrocarbons"), and in this bound form they penetrate into the cell nucleus, where they can modulate the action of certain genes and influence the way enzymes are synthesized (1, 8).

[1]) Dr. Ehrenstorfer, Gögginger Str. 78, 8900 Augsburg, West-Germany

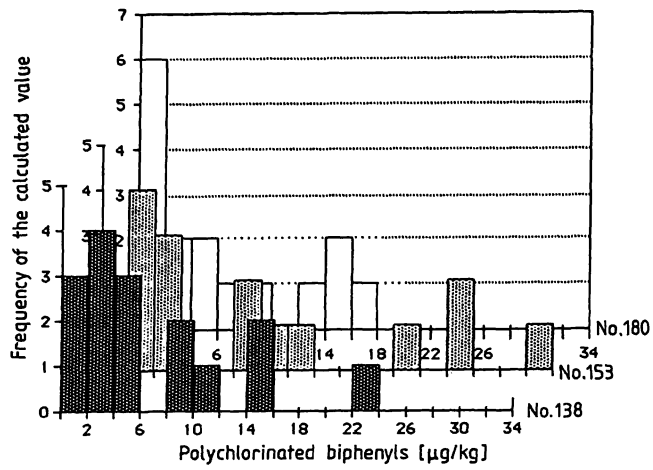

Fig. 3. The pattern of the individual concentration levels for polychlorinated biphenyls:
▨ no. 138 = 2,2',3,4,4'5'-hexachlorobiphenyl,
▩ no. 153 = 2,2',4,4',5,5'-hexachlorobiphenyl, and
□ no. 180 = 2,2',3,4,4',5,5'-heptachlorobiphenyl
in 16 sperm fluid samples. The levels have been protracted in the X-axis with a class range of 2 µg/kg. The Y-axis indicates the frequency of the calculated value.

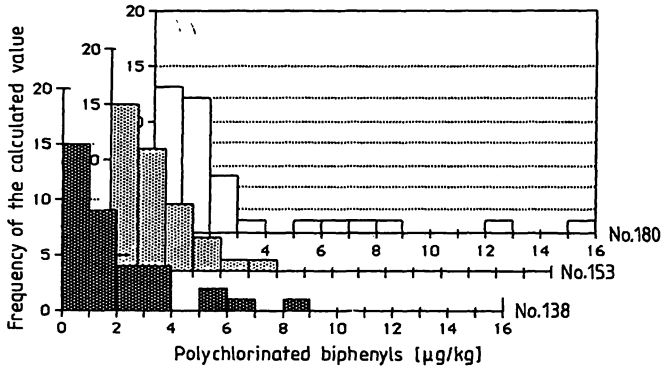

Fig. 4. The pattern of the individual concentration levels
▨ no. 138 = 2,2',3,4,4'5'-hexachlorobiphenyl,
▩ no. 153 = 2,2',4,4',5,5'-hexachlorobiphenyl, and
□ no. 180 = 2,2',3,4,4',5,5'-heptachlorobiphenyl
in 37 follicular fluid samples. The levels have been protracted in the X-axis with a class range of 2 µg/kg. The Y-axis indicates the frequency of the calculated value.

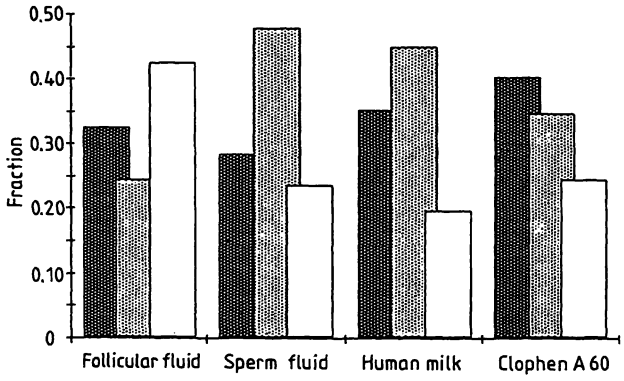

Fig. 5. Pattern of the 3 main congeners in follicular and sperm fluid, human milk, and a commercial mixture (Clophen A 60) according to their fractions. The concentration of the 3 substances was set at 1.00:
▨ no. 138, = 2,2',3,4,4',5'-hexachlorobiphenyl)
▩ no. 153, = 2,2',4,4',5,5'-hexachlorobiphenyl)
□ no. 180 = 2,2',3,4,4',5,5'-heptachlorobiphenyl)

Polychlorinated biphenyls with high environmental persistence do not possess adjacent, unsubstituted carbohydron atoms, like polychlorinated biphenyls No. 153 and No. 180 (8).

In the same way, highly chlorinated polychlorinated biphenyls containing a 2,3,4-trichlorophenyl group (like No. 138), can resist metabolic breakdown and so accumulate very rapidly in body tissue (8).

The environmental samples were all characterized by a pattern different from that of the commercial polychlorinated biphenyl formulation Clophen A 60 (see fig. 5). Variable resistance to metabolic breakdown leads to a new distribution pattern of the congeners in relation to each other.

It is striking, however, that in follicular fluids a tendency exists to accumulate the more highly chlorinated components, as can be seen for heptachlorobiphenyl No. 180. Also, in the accumulation of both hexachlorobiphenyls, No. 138 and 153, one can see that the accumulation patterns for follicular and sperm fluid are mirror images of each other.

Reasons for this difference are to be found in the way that the two products distribute in the body:

The sperm plasma consists of diverse secretions from accessory reproductive organs such as secretions from the testicles, the epididymis, the seminal duct, the seminal vesicle, the prostate, *Cowper*'s and *Littré*'s glands. The content of the individual factors can be estimated from their amount of acid phosphatase, fructose and spermatozoa (26).

Follicular fluid, on the other hand, is only partly made up of an active secretion from the follicle cells; the other part originates from the liquefaction of whole cell formations. In this sense its composition is micro- and macroscopically similar to serum, as the basement membrane is also very permeable to low and high-molecular substances that can fuse into the fluid in a matter of minutes (26–28). The different modes of origin and the different lipid contents probably lead to selective accumulation, since the lipid-soluble pesticides are transported mainly by lipoprotein fractions in the blood (3).

The lipids in the seminal fluid are derived, for the most part, from the prostate, and they contain mainly phospholipids (2 g/l) and cholesterol (1 g/l); free fatty acids and prostaglandins make up only 10% of the total lipid content (29).

It is not yet possible to deduce the total level of contamination with polychlorinated biphenyls from the concentration levels of 3 main congeners in follicular and sperm fluid. A variable accumulation pattern

seems to exist and not all of the individual congeners have been determined (18, 19).

Certainly different accumulation patterns can affect the toxic potential of the polychlorinated biphenyls. As shown in the present work, the proportion of the more highly chlorinated coplanar compounds may be increased in body compartments associated with reproduction.

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