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Reproductive Toxicology - The Science Today

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DR. CARTHEW: Okay, the first speaker is Professor Dieter Neubert, from the Institute of Chemical Pharmacology and Toxicology where he has been Director for 25 years. It is the Benjamin Franklin Medical Center, the Free University of Berlin, and he has published over 500 publications which I think gives him some considerable credibility in the introductory talk which is going to be reproductive toxicology, the science today. Would you like to come up?

PROF. DR. NEUBERT: Mr. Chairman, ladies and gentlemen, thank you for the kind invitation to the Toxicology Forum. It is always nice, again, to be here at the Toxicology Forum because there are so many interesting discussions.

[The following is a manuscript provided by Dr. Neubert. The actual question and answer session is included at the end.]

Summary

Reproductive Toxicology is concerned with chemical or physical agents interfering with *fertility* in both gender. Adverse effects may be induced *directly*, especially in adult males by damaging the semen producing epithelium (example: DBCP), or *indirectly*, predominantly by interfering with *sex hormonal* homeostasis in both gender.

Several *male developmental* processes critically depend on inducing influences of *androgens*, and these processes may be (at least theoretically) disturbed by interfering agents, provided that the exposure is high enough. The processes possibly susceptible include: (**1**) androgen-dependent differentiation of the *male phenotype* during late embryonic development. (**2**) Dihydrotestosterone-dependent differentiation of the *male secondary sex organs* during the fetal period. (**3**) Formation of a fixed number of *Sertoli cells* during the perinatal period. (**4**) Imprinting of *male sexual behavior* in defined *brain* areas during the perinatal period. (**5**) Imprinting of the *pulsatile* GnRH *regulation* of hypophysial hormone formation in *both* gender via the *hypothalamico-hypophysial axis*.

Most of these processes *cannot* made-up for at later developmental stages, and resulting defects are largely irreversible. Defects are known to be inducible experimentally by anti-androgens or possibly by a large excess of estrogens (by disturbing the balance between male and female sex hormones). Several of the processes described for male development can also be altered in *females* by exposure to a large excess of male sex hormones.

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Many adverse effects on reproductive functions can also be induced in the adult organism. Alteration of the feedback mechanism guaranteeing homeostasis is one mode of action. Many synthetic steroid compounds exhibit effects on *more* than one *receptor*, thus causing a very *complex* result. This must be taken into account when analyzing possible potentials of "environmental" agents assumed to interfere with sex hormonal actions.

Many adverse hormonal actions are *well established* from experience in experimental and clinical *medicine*, using either natural or synthetic sex hormones, or specific enzyme inhibitors. In contrast, possible effects of "environmental" agents either mimicking or inhibiting sex hormonal actions are less well studied in clinical trials. Because of species differences in hormonal effects, and especially considerable species differences in pharmacokinetics, data of *animal studies* are of rather *limited* predictive *value* for quantitative *extrapolations* in preventive hazard minimization (but may be useful for revealing possible mechanisms of action). Data of *in-vitro* studies are even less suitable for extrapolations. It may be doubted that exposure of the *general population* to induce clear-cut clinical effects. Certain adverse effects induced by e.g. greatly unbalanced diets or after accidental overdoses cannot be excluded.

1. Introduction

In the Anglo-American scientific literature, *Reproductive Toxicology* is distinguished from *Developmental Toxicology*. In this presentation, main emphasis is laid on impaired *reproduction*, which means interference with *fertility*, i.e. providing the basis for producing the next generation. Fertility with respect to *both* gender is involved in successful reproduction.

Reproductive Toxicology is a rather complex toxicological issue, with many facets, various endpoints involved and many different mechanisms of action.

The following aspects must be considered when attempting to assess possible adverse health effects in the area of reproductive toxicology:

- ❖ Sources of information
  - Animal *versus* human data, hazard *versus* risk
  - *In-vivo versus in-vitro*
  - Female *versus* male contribution.
- ❖ Types of possible interferences
  - Direct interference with structures and functions required for reproduction
  - Indirect interference (e.g. mimicking or inhibiting hormonal functions).
- ❖ Susceptible periods during development
  - Pre-, /peri-, /post-natal development
- ❖ Classification of interfering agents
  - Direct effects on sex hormone receptors
  - Indirect effects on sex hormonal systems
- ❖ Special aspects
  - Interference with very specific *versus* multiple receptors
  - Dose-response relationships

Some aspects of the vulnerability of the (sex hormonal) endocrine system to xenobiotic influence have been discussed elsewhere (Neubert 1997), and the figures presented here have been published before in that paper.

2. Sources of information

Except with relevance for humans, reproductive toxicology may, in principle, may be of interest to some problems of the live-stock, and even certain aspects of wildlife and environmental toxicology.

2.1 Animal *versus* human data, *in-vivo* *versus* *in-vitro* data

In toxicology, data of *in-vivo* studies always have preference over those of *in-vitro* experiments, and results of good studies in *humans* always have a much higher predictive value than data of *animal* studies. Reproductive toxicology is no exception in this respect.

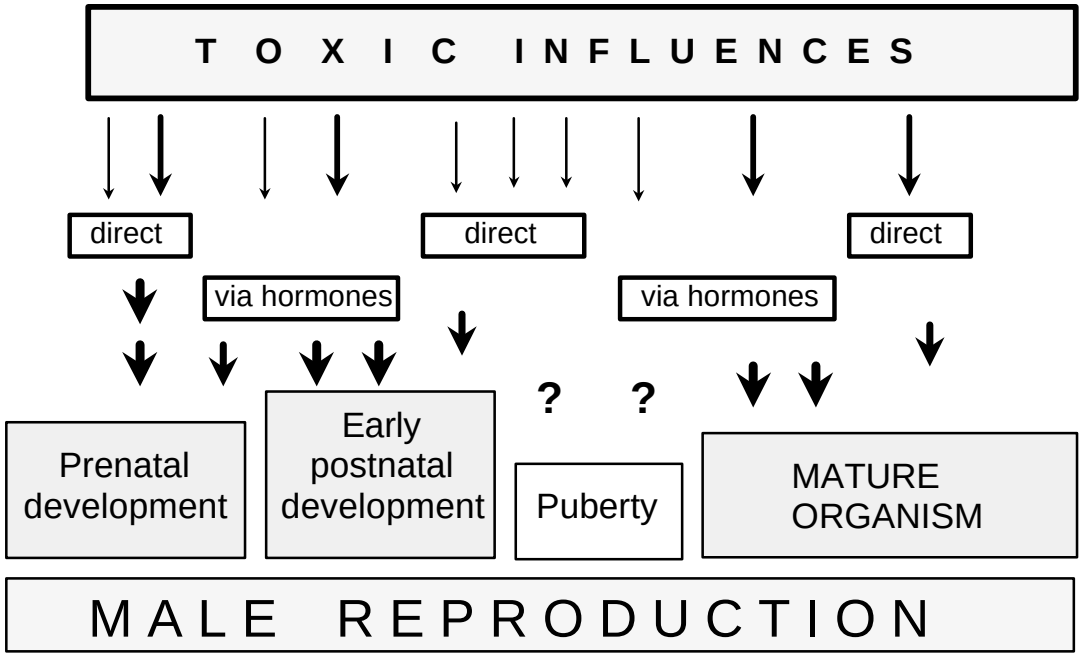

Figure 1: Periods susceptible to interference with fertility. Fertility may be influenced during many pre- as well as postnatal developmental periods. An adverse effect may be induced directly (by a direct toxic interference with reproductive organs or functions) or indirectly (predominantly by modifying hormonal contributions essential for reproduction).

2.1.1 Risk assessment versus preventive hazard minimization

Human risk[b] assessment, which is assessing the incidence of an adverse effect at a defined dose or exposure (and which can *exclusively* be done based on *human* data), and preventive hazard minimization, which is *extrapolation* from animal or *in-vitro* data, have very little in common. The intentions, methodology, and the predictive value are drastically different (*see:*

[b] Toxicologic "**risk**" is defined as: *incidence in humans at a defined dose or exposure*. This is a dose-response relationship. Thus, "risk" is a value. Expressions such as "risk prevention" or "risk management" are nonsense, because a value can neither be prevented nor "managed". What is meat is a **hazard** (this [which designates a more or less realistic **possibility**] can be prevented and even "managed").

Neubert 1999). Mixing-up the terminology has greatly contributed to misinterpretations among scientists as well as considerable misunderstanding and confusion within the public.

2.2 Female *versus* male contribution

Impaired fertility may be induced in both gender. Although by extensive use of hormonal contraceptives predominantly female fertility has been affected, recent attention has mainly focussed on possible effects on the male population. The rationale is that numerous developmental processes might be susceptible to interference.

3. Types of possible interferences

One may distinguish *direct* interference of agents with various structures and functions involved in reproduction from *indirect* acting agents (figure 1). Both types of substances interfere in various ways with actions of sex hormones, either by mimicking or exaggerating effects of these hormones, or by inhibiting the functions and disturbing homeostasis.

3.1 Direct interference with structures or functions required for reproduction

Numerous agents have been found to directly interfere with *male* reproduction in *animal* studies, mostly by damaging the semen producing tissue. In contrast, only few groups of agents have convincingly been demonstrated to affect *human* male reproduction. Such agents include some heavy metals, some cytostatic agents, and a few pesticides. Even fewer substances were shown to directly interfere with female reproduction.

One of the best examples of interfering with testicular structure and function in humans is the effect of DBCP (*1,2-Dibromo-3-chloropropane*), which had been used as a nematocide in agriculture (Lipshultz et al. 1980).

3.2 Indirect interference (e.g. mimicking or inhibiting hormonal functions)

Recent attention has focussed on possible adverse effects on fertility by chemicals *mimicking* or *inhibiting* functions of *sex hormones*. Although adverse effects might also be expected to be inducible in women at excessive exposures, the possibility of an interference with *male* fertility has predominantly been discussed (e.g. Ashby et al. 1997, Daston et al. 1997).

The *male* reproductive system is especially interesting, because there are defined periods during development during which androgens are essential for male differentiation (figure 2).

4. Susceptible periods during development

Embryonic development initially passes through an *indifferent* period, during which *no* differences with respect to *gender* are recognizable (but of course the genotype of the embryo is determined [humans: 44+XX or 44+XY]). In humans, this indifferent period starts within the fifth week with the migration of the primordial germ cells from the yolk sac epithelium to

the genital ridges. During this period, the male embryo is not capable of producing androgens. Interference with this early development is only possible by toxic (or mutagenic) direct influences. If the primordial germ cells fail to reach the genital ridges within the critical period, no gonads can develop.

The gender-specific developmental period begins with the formation of the *primitive sex cords* during the sixth week of gestation (figure 3). The *male phenotype* is differentiated under the influence of the Y-chromosome, and the *primitive testes cords* (seminiferous *tubules* with a lumen are not formed before puberty) are formed.

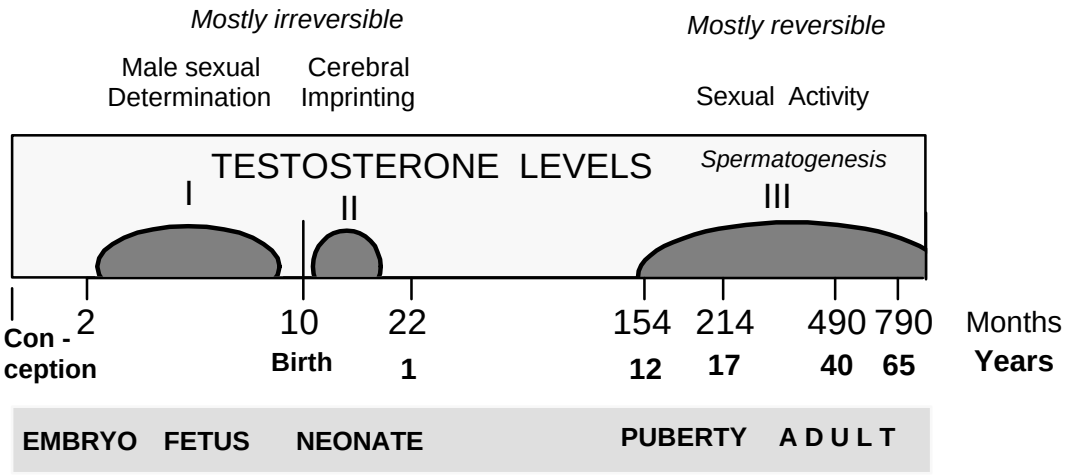

Figure 2: Androgen surges during development. There are several periods during pre- or early postnatal development in which levels of androgens are critical. If insufficient levels of the androgens are provided or androgen actions are counteracted, normal male development cannot be achieved.

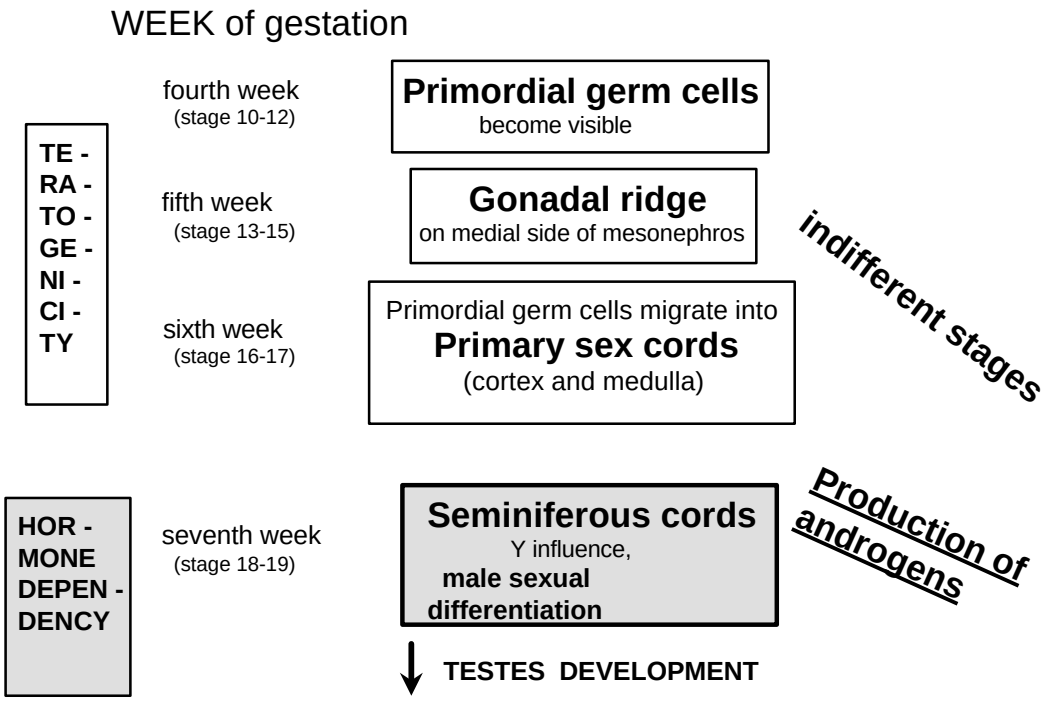

Figure 3: The embryonic development of the sex organs proceeds indifferently until the *sixth week* of gestation. The male or female phenotype cannot be distinguished (but of course the genotype is typical). During the *seventh week* of gestation (developmental stages 18-19 in primates) *seminiferous cords* (a lumen is formed not before puberty) differentiate under the influence of androgens secreted by the fetal Leydig cells.

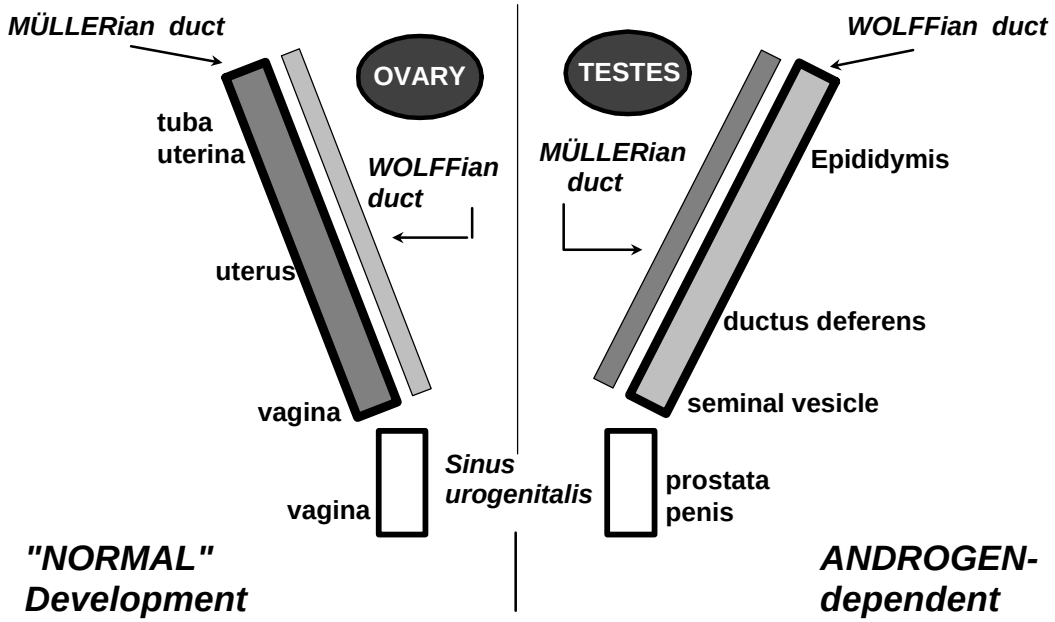

Figure 4: Differentiation of the *Müller*ian and the *Wolff*ian ducts to the female or male genitalia. While no fetal sex hormones are required for female development (apparently female hormones from the mother and the placenta are sufficient), male differentiation (i.e. differentiation of the Wolffian and regression of the Müllerian duct) can only be initiated by a sufficient amount of fetal androgens.

4.1 Pre-, peri-, and post-natal development

There are at least 3 critical *androgen-dependent* processes (possibly 4) occurring during *male* development, but several of these processes may also be disturbed in the developing *female* fetus, if it is exposed *in utero* to high enough doses of androgens or anti-estrogens.

4.1.1 Fetal development of male genital duct system and the external genitalia

Within the *primitive testes cords*, in human fetuses predominantly during the 4th to 6th month of gestation, also (interstitial) *Leydig cells* develop, which are capable of producing *androgens*. These hormones (specifically 5-alpha-dihydrotestosterone) are essential for the formation of the male *genital duct system* and the *external genitalia* during the fetal period. Influenced by the androgens, the *Müller*ian duct regresses, and the *Wolff*ian duct differentiates to the male external sex organs (figure 4). We must remember that the "normal" development (i.e. regression of the *Wolff*ian [mesonephric] and differentiation of the *Müller*ian [paramesonephric] duct) is to a *female* organism (figure 4), and this phenotype will result in the male genotype if the androgen surge fails to occur, or this hormone-dependent process is counteracted. For the differentiation of the female sex organs apparently no specific hormones produced by the conceptus are necessary, and the estrogens required for differentiation (if there is any need) will be provided by the mother and the placenta.

Table 1:
Periods susceptible to interference during pre- and post-natal development

Developmental Period	Gender	Effect
embryonic	both	Unspecific gonadal development
fetal	male	Induction of male secondary sex organs
	male	Formation of Sertoli cells (fixed number)
perinatal	both	Imprinting of regulatory hypothalamico/hypophysial system
	male (female?)	Imprinting of sexual behavior (predominantly in males)
puberty	both	Strong hormonal influence on development
adult organism	both	Effects on various sexual functions

The susceptible period may vary somewhat in different species.

These developmental processes and the contribution of hormones have been well studied in animal experiments, and the results are also confirmed in humans by a number of well-defined diseases.

4.1.2 Late fetal and early postnatal male development

At least *three* additional important processes occur during *male* development at the late fetal and the early postnatal periods (table 1). Some of these developments also concern the *female* organism. These processes during development are designated as "*imprinting*", which means they cannot made up for later, they are predominantly *irreversible*. Most of the information on these processes comes from rodent studies, but they may also be relevant for humans.

(1) *Sertoli* (sustentacular) *cells* exhibit an important role with resect to the maturation of sperm within the testes. The *number* of spermatozoa within the ejaculate critically depends on the *number* of Sertoli cells present in the testes. These Sertoli cells are formed prenatally, and the number fixed prenatally apparently cannot be changed during late postnatal life. Although these processes have been investigated extensively in rodents (Orth 1982), the contribution of androgens is not clear, and the relevance for humans has not yet been confirmed by human data (although a similar situation as in rodents may be expected).

(2) Of significance is a possible interference with the imprinting of the *pulsatile* GnRH and gonadotropin *discharge* from the hypothalamus, which is essential for a normal sex hormonal regulation (by FSH and LH) for the rest of the life in both gender. For the proper regulatory function of the hypothalamico-hypophysial axis a *pulsatile* signal is required, otherwise

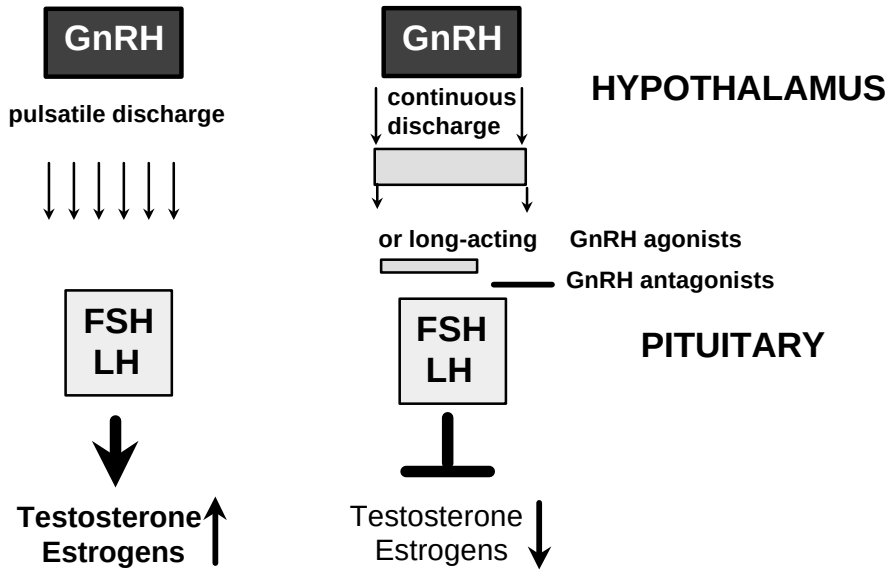

Figure 5: A pulsatile signal from the hypothalamus (GnRH) is required for the regulation of the secretion of FSH/LH from the hypophysis. These hormones are necessary for the formation of sex hormones within the gonads and the release of these hormones into the periphery. The imprinting of this *pulsatile* regulation takes place during the perinatal period. A continuous signal does not induce a regular FSH/LH secretion, and hormonal dysfunction results.

no normal sperm formation and no regular ovarian cycle is possible (figure 5). In by now classical experimental studies, it was shown that anti-androgens can inhibit this imprinting in *male* rats, resulting in irreversible effects when applied during a period of shortly before birth to the 10th day postnatally (Neumann and Steinbeck 1974). No adverse effect could be induced at later developmental stages. Similar irreversible effects (permanent estrus and sterility) were induced in *female* rats by treatment with androgens.

(3) It has also been known for a long time from experimental studies that typical *male sexual behavior* is imprinted by sex hormones during the perinatal period (the susceptible period possibly being somewhat variable in different species). One location responsible for such an imprinting in rats has been suggested to be the *medial preoptic area* in the brain, this area being larger in males than in females. Early castration or treatment with opposite sex hormones changed the size of this area in a typical way, and this susceptibility was confined to a certain perinatal period (Gorski et al. 1980). Conclusive studies on primates have not yet been performed. If the classical and often repeated results in rodents are relevant for humans, this would provide evidence that homosexuality must be considered as a congenital disease or defect.

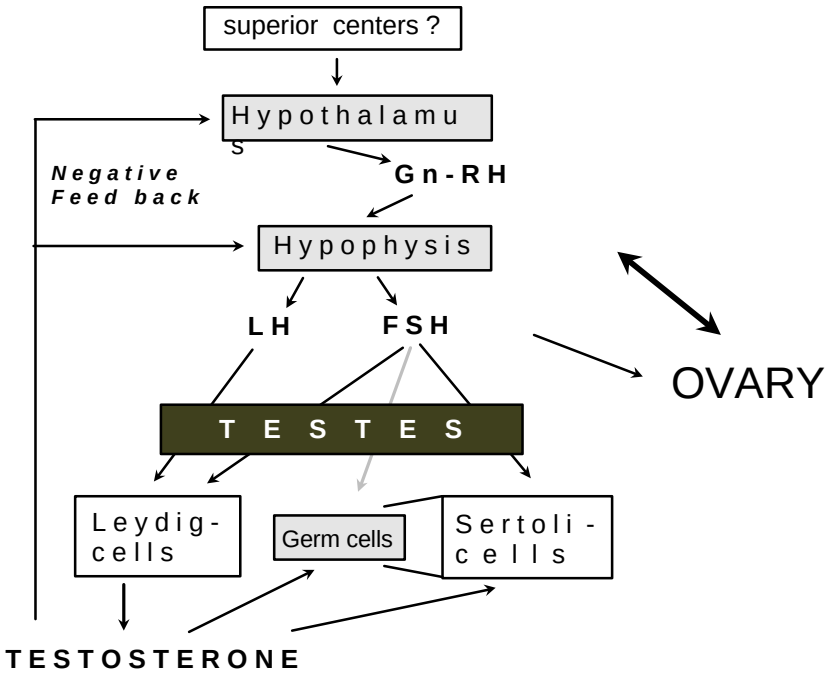

Figure 6: The extensive feedback regulation is an important aspect of the sex hormone function during the entire life in both gender. Many agents may interfere rather with this regulatory feedback process than directly with the sex hormone receptors. Often it is a matter of the dose which mechanism (the feedback regulation or the direct effect on peripheral receptors) is more susceptible.

4.2 Effects inducible during late postnatal life

Puberty is a very important period for hormone-mediated maturation processes, both with respect to organ development and behavioral changes. Surprisingly little information on chemicals possibly affecting these processes is available. One reason may be that puberty extends over a rather long period, and possible interference with development is short and modifications induced are likely to be reversible. The chance of reversibility in the adult organism seems to be an important aspect with respect to possible interference with sex hormonal systems (e.g. hormonal contraception).

Besides direct effect effects on *receptors*, interference with *feedback* regulatory mechanisms plays an important role (figure 6). Experience with numerous steroidal and non-steroidal substances used in experimental and clinical medical research points to a considerable complexity in this area of research. This must be taken into account when attempting an assessment of hormone-like actions of chemicals. Although temping, a simple classification into "estrogens" etc. provides quite unsatisfactory information. Several substances, although exhibiting an affinity to sex hormone receptors, also act indirectly and thereby even in an opposite way. An example is the non-steroidal anti-estrogen *clomiphen*, which stimulates spermatogenesis and facilitates ovulation, via counteracting the negative feedback regulation by endogenous estrogens.

Table 2:
Classification of some types of agents interfering with fertility

Agents with an apparent *direct* action on reproductive functions (e.g. DBCP)

Agents with an *indirect* action on reproductive functions

Agents interfering with development or acting on the adult organism

Agents interfering with receptors of sex hormones
androgens – anti-androgens,
estrogens – anti-estrogens,
progestins – anti-progestins,
agents with mixed functions,

Agents interfering with formation and functions of sex hormones
agents interfering with the formation of sex hormones
agents interfering with other hormones (e.g. thyroid hormones)
agents interfering with other regulatory systems (e.g. dopamine)
agents interfering with certain CNS functions (libido, erection, etc.)

5. Classification of interfering agents

There is *ample information* available in medicine from clinical as well as experimental studies on *natural* or *synthetic steroid* and on certain *non-steroid* substances with sex hormonal functions. In fact, many of such substances are routinely administered to millions of women (e.g. as hormonal contraceptive treatment), or have been applied during pregnancy. In case of the latter, retrospectively recognized as useless, treatment for imminent abortion, extensive data on exposure to *diethylstilbestrol* (DES) have been gathered (e.g. Herbst et al. 1977, Bibbo et al. 1977). Altogether, we must recall that we are not moving within no man's land, but in many respects within a medical field with ample experience.

From a pragmatic point of view, it may be prudent to classify substances according to different mode of actions (table 2). However, we must remember that the situation is much more complex, and simple classifications may be misleading, because many substances will exhibit more than one effect. Furthermore, secondary changes are bound to occur, when the homeostasis is disturbed by drastically changing the concentration of one component.

5.1 Direct effects on sex hormonal receptors

Many substances have been synthesized in an attempt to obtain drugs with well defined actions, and preferentially those being effective at the oral route. While some of such substances are modified steroid hormones, others show a chemical structure which may not predict specific hormonal effects. Besides well studied drugs such as DES, many "*ecohormones*" or "*xenohormones*" belong into this category. The substances have been reported to exhibit a *direct* action (either agonistic or antagonistic) on various (table 2) sex hormone receptors.

DDT is a classical example of such a substance with direct sex hormonal properties, as observed in several experimental systems. DDT and its metabolite *o,p'*-DDT have long been con-

sidered as "pure estrogenic agonist" (Galand et al. 1987). However, more recently it was reported that another metabolite of DDT (*p,p'*-DDE) exhibits, at least *in vitro*, a strong *anti-androgenic* potential (Kelce et al. 1995). This underlines the fact how complex a situation may be, and of course there is *no clue* on a possible relevance of these data for an exposure situation in humans.

5.2 Indirect effects on sex hormonal actions

Besides direct actions on corresponding sex hormonal receptors, there are many possibilities to *indirectly* interfere with such hormonal actions (table **2**). There are predominantly two mechanisms which may be of significance: (a) an interference with the *regulation* of the formation and secretion of sex hormones, and (b) an interference with the *metabolism* of hormone formation, leading either to *decreased* or *increased* levels of the natural hormones. There are ample examples, from clinical as well as experimental medicine, for these two mechanisms. The *feedback* mechanisms of the hypothalamico-hypophysial axis is a well-known mechanisms for interfering with sex hormonal regulation by FSH and LH. Besides inhibitions induced by all natural hormones, also *stimulation* of this regulatory mechanism is established (e.g. by *clomiphene*, which in a simple receptor test would rather show-up as anti-estrogen). Several inhibitors of sex hormonal *metabolism* (e.g. inhibitors of 5-alpha-reductase) also have been synthesized and are therapeutically used (e.g. *finasteride*). The drastic interference with the male sex hormonal system, and especially the *specific teratogenic* risk (Clark et al. 1993), would also not be recognized in a simple receptor test.

5.3 Indirect effects via other hormonal systems

Indirect effects on *sex hormonal* functions are not confined to interference with mechanisms directly connected with the sex hormonal systems. Well-known are effects on *prolactin* secretory regulation, which is under *dopamine* influence (figure 7). Therefore, all dopamine-agonists and -antagonists (e.g. *neuroleptic* substances), at appropriate doses, may be expected to exhibit an effect on prolactin secretion, and thereby indirectly on certain sex hormonal functions.

Another hormonal system reported to be associated with certain sex hormonal functions is the *thyroid*. Pre- and perinatal alterations in thyroid function may affect the formation of *Sertoli* cells, and thereby *sperm* formation in later life (van Haaster et al. 1993) in experimental systems. The relevance for humans is unknown.

6. Special aspects

There are some special aspects which have been reported with respect to some of the agents inducing hormonal dysfunctions. These relate to the specificity of the peripheral receptors, special dose-response relationships, and the question of the relevance of chemically-induced dysfunctions for humans.

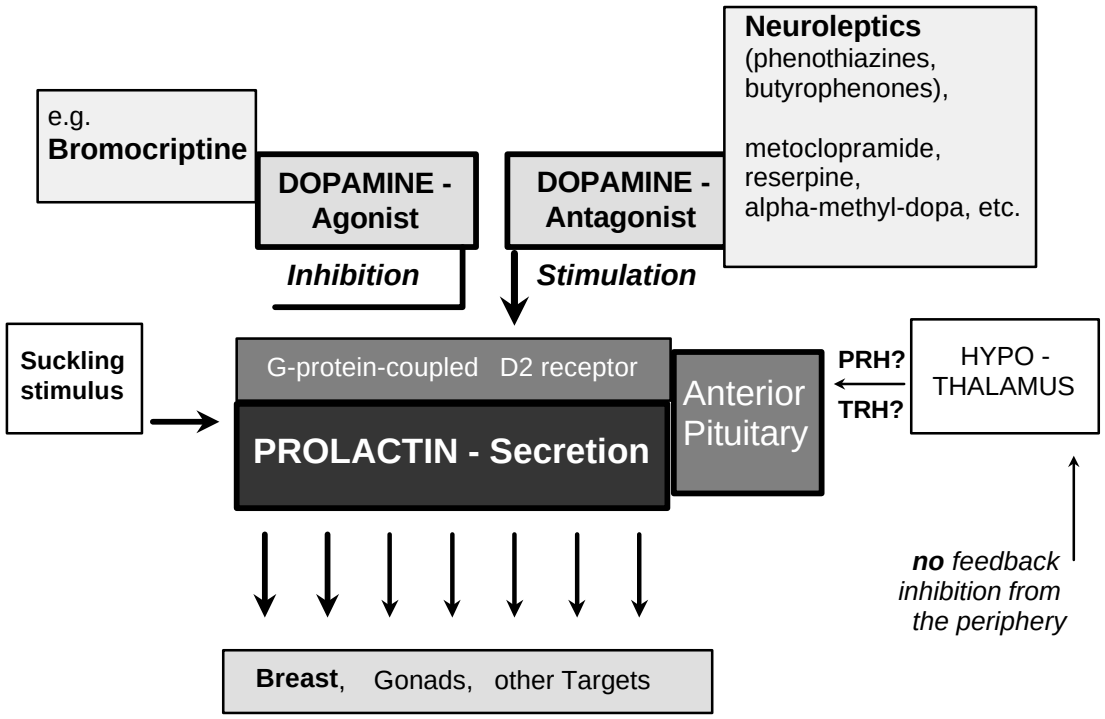

Figure 7: Regulation of the *prolactin* secretion, and its *indirect* effect on sex organs and sexual functions. The secretion is regulated by a dopamine-dependent process. Therefore, substances acting on the dopamine system either as agonist or antagonist may alter the regulatory process. There is no feedback regulation, e.g. by the sex hormones.

6.1 Interference with very specific *versus* multiple receptors

From the vast experimental and clinical evidence accumulated from research on modified steroid hormones, it is obvious that many of such synthetic substances exhibit effects on *more than one* sex hormone receptor. Many of the progestins used in hormonal anticonceptive drugs e.g. exhibit androgenic properties, and even the steroidal aldosterone antagonist spironolactone, primarily having an affinity to quite another hormonal system, may cause several adverse effects on sexual functions.

6.2 Dose-response relationships

There have been claims in the literature that under certain experimental conditions only small doses of substances with a potential for inducing sex hormonal dysfunction exhibit "ad-

Table 3:
Other factors responsible for infertility

Contraception.
Infections of the genital tract (especially in women, and when poorly treated).
Psychological factors (in both gender).
Malformations of the genital tract (especially in women).
Complications during pregnancy (e.g. multiple abortions).

verse" effects, but not higher doses, and that no NOAEL (no-observed-adverse-effect-level) might exist. As a unique feature it was postulated that no dose should exists which induces no effect.

In the majority of cases such claims have not been backed-up by proper data on dose-response relationships, which is a minimum requirement for making such odd statements. The situation is especially obscure when the "effect" is observed in a special mouse strain only, and can hardly reproduced in another strain or in other laboratories. One should not seriously discuss such "effects" unless sufficient data on dose-response (down to a NOAEL) are provided. Another important aspect is the endpoint involved. Effects on anogenital distance or prostate weight in rodents will have much less weight of evidence than data on overall fertility (which will be unimpaired in almost all these cases).

It should be remembered that dose-response curves cannot always be expected to be linear (even in the usual form of e.g.: probit of effect *versus* log of the dose) or S-shaped, especially when complex mechanisms of action are involved, as often is the case with chemically-induced sex hormonal dysfunctions. "U"- or "J"-shaped curves are well established in toxicology for many situations (and are also well-known in pharmacology). Par example, for different effects of TCDD, including tumor promotion, effects on the immune system, and effects of other chemicals on many other end points such dose-response curves have been published. It is essential to disclose the reasons for such a dose-response, i.e. analyzing the components and mechanisms involved in the complex reaction. No properly analyzed and reproducible case is known in which no NOAEL could be established (if such an "effect" should occur, one should confirm the validity of the reference group! and repeat the study several times). If the "effect" cannot be reproduced in other laboratories, it should not be considered as real.

6.3 Other factors known to affect fertility

It would be wrong to assume that *chemicals* capable of inducing sex hormonal dysfunction are the *main cause* of infertility. Some of the main factors, well-known in Obstetrics/Gynecology are compiled in table **3**.

7. Conclusions

According to the present state of the art, there are three questions which must be answered when attempting to evaluate dysfunctions within the sex hormonal system possibly induced by xenobiotics:

1. What is the relevance of data obtained from animal and *in-vitro* experiments for the situation probably existing in humans?
2. Are the presently applied experimental test methods adequate to reveal relevant hazards probably existing for humans, or are additional end points to be considered in test systems?
3. Do hormonal dysfunctions, induced by environmental agents, really pose a hazard for the general human population (or for some especially exposed subpopulations [if: yes, for which subgroup?]), justifying extensive additional testing?

Based on our present state of scientific knowledge, some limited answers are possible on these questions. Interestingly, the conclusions do not seem to have changed significantly from those presented almost 5 years ago (Neubert 1997), despite considerable experimental efforts:

Ad (1): Especially in the area to be discussed here, data of animal experiments (or even more those of *in-vitro* studies) are of very limited use for extrapolations to a situation probably relevant for humans. There are a number of crucial *limitations* which include the following:

- Actions of sex hormones are *complex*. Effects induced by one sex hormone must be evaluated in the context of the homeostasis of sex hormones as well as within the concert with many additional factors. Many of the sex hormonal effects and those of the other factors exhibit pronounced species specificity. This holds especially true for actions during pregnancy and during the early postnatal period. Without profound knowledge of this species specificity no valid extrapolation from one species to another (and especially from rodents to primates) is meaningful.
- For any useful extrapolation to the situation possibly existing in humans, data of *in-vitro* studies on sex hormonal dysfunction alone are rather worthless. Almost no conclusions on possible effects *in-vivo* can be drawn without additional pharmacodynamic as well as pharmacokinetic information.
- For any useful *extrapolation between species*, *pharmacokinetic* data are essential. Extrapolation on the basis of doses is no longer scientifically justified. Very few kinetic data have been provided *together* with animal studies in this area of research, and data on pharmacokinetics and metabolism in humans are regularly missing with respect to relevant human exposure. For these reasons, attempts of preventive hazard minimization must remain largely speculative. Such highly speculative (largely political) measures may also be taken with a minimum of scientific information (they are largely *not* based on scientific data anyhow).

Ad (2): Experimental test methods presently used for routinely assessing effects on fertility include either *segment I*-tests, or *multigeneration* studies (with *modifications* for better assessing hormonal dysfunctions suggested by several individuals [including ourselves] and regulatory institutions). These rather long-term studies provide reasonable information on possible adverse effects on *overall fertility* in the species studied and the experimental conditions chosen. It may become more difficult (especially in the

multigeneration test used regularly for "environmental chemicals") to *localize* any defect occurring within the sequence of the many events. Because of the *complex nature* of the effects to be expected, routinely performed much more specific studies (which would have to be manifold) do not seem to be practical.

Ad (3): Fertility, generally is operating with a large access (of sperm, of oocytes, of hormone levels, etc.), or a large "safety margin". Small changes in the majority of aspects involved do *not* induce deficits in overall fertility. Furthermore, an important discrepancy intrinsic to animal studies must be remembered: most routinely performed animal tests are conducted by highly *overdosing* the chemicals to be tested. In contrast, especially exposure of the general population to "environmental chemicals" is very *low*. A possibly hazardous agent must be either rather *potent*, or should accumulate to a high *concentration* within specific organs of the human organism, in order to induce relevant adverse effects. This is unlikely for the majority of agents with some potential for inducing sex hormonal dysfunctions.

From a scientific point of view and considering the many possible mechanisms of action, which with respect to humans are still ill-understood, it is not doubtful that induction of hormonal dysfunction by chemicals is an interesting area of research. More information is needed on the relevance of e.g. perinatal imprinting of male (and female) behavior in the brain of primates. On the other hand, it seems justified to conclude that the hazard to reduce overall fertility in humans by chemicals inducing sex hormonal dysfunction is small, if it exists at all. Not numerous routine studies are needed, but research to better understand the essential processes essential for an undisturbed fertility in *primates*.

8. References

Because of the huge number of publications in this field of research, only a small selection of references is provided.

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DR. CARTHEW: Thank you very much. That was an excellent overview. Are there any questions? Please state your name?

DR. DASTON: George Daston, Procter & Gamble. Dr. Neubert, I think it is an important and perhaps essential point that you made that we need more human data. What is the state of, at least in Europe, I guess of collecting data on birth defects?

PROF. DR. NEUBERT: George, as you know, that is a very difficult issue because we have some systems, but most of the systems are not really reliable, and they are especially not reliable if you are asking for dysfunctions. We are measuring morphological defects in general, but we are not measuring dysfunctions, and you are talking of dysfunctions, and as you know there are many more dysfunctions, possibly of the immune system or some others, and I think there are very few data on this.

It is difficult to do anyhow, but I think almost no attempts have been done so far.

DR. CARTHEW: Another question? Dr. Cabral?

DR. CABRAL: You mentioned the formation of the Sertoli's cells. You said that they cannot be changed in later life. This was making reference to rodents or to humans, alike?

PROF. DR. NEUBERT: This is mostly studied in rodents, and we have very little information in humans in this respect, but in rodents I think it has been shown that at birth the number is fixed, and it does not change, and of course, you know that Sertoli's cells are very important for sperm formation. So, the number of sperm is fixed and is reduced if the number of Sertoli's cells is small.

DR. EMERSON: Emerson, from Atlanta. Just a comment, and that is a classical example of surveillance and intervention is that of neural tube defects and the fortification with folic acid, and of course the question has been is intervention effective, and the latest study that, the latest and only study that has really looked at that in the US population indicates that with fortification there is a 19 percent reduction in neural tube defects in humans, and this is based on human data, and I think this is the approach that we need more of in this cloudy area of endocrine disrupters.

PROF. DR. NEUBERT: Thank you very much. I think that is a very good comment, but of course you have to realize that these are not chemically induced abnormalities, but they are spontaneous neural tube defects.

DR. CARTHEW: Are there any other questions?

Thank you very much, Professor Neubert. That was wonderful.

(Applause.)