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Endocrine disruption and human health: Workshop report on the state of the art

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Summary

Within the project on endocrine disruption and human health one aim is to keep track with the state of the art as regards endocrine disrupter research relating to human health effects. This report summarises the presentations given at the Workshop on the subject held in May 2000 in Copenhagen, which was organised by Professor Niels Skakkebaek. The workshop brought together a considerable group of key scientists in the field. Both timely overviews as well as current research results were presented. The workshop concluded that endocrine disruption remains a matter of concern, although causal relationships between exposure to environmental endocrine disrupters and human health effects have not been proven to date. Particularly, human exposure data are needed to found a solid basis for actual human risk assessment in the field of endocrine disruption.

Samenvatting

In het kader van het project ‘endocriene verstoring humaan’ worden onder meer actuele ontwikkelingen gerapporteerd over de relatie tussen endocriene verstoring en humane effecten. Dit rapport geeft een overzicht van de presentaties die over dit onderwerp gegeven werden tijdens een workshop, gehouden in mei 2000 in Kopenhagen, die werd georganiseerd door prof. Niels Skakkebaek. De workshop bracht een niet onaanzienlijke groep expert wetenschappers op dit terrein bijeen. Actuele overzichtsverhalen zowel als nieuwe resultaten van lopende researchprojecten werden gepresenteerd. De workshop concludeerde dat endocriene verstoring een onderwerp van zorg blijft, hoewel causale relaties tussen blootstelling aan endocriene stoffen via het milieu en gezondheidseffecten bij de mens tot op heden niet zijn aangetoond. Er zijn met name meer humane blootstellingsgegevens nodig als basis voor een actuele risicoschatting voor de mens met betrekking tot endocriene verstoring.

1. General Introduction

From 27-30 May 2000 in Copenhagen, Denmark, a workshop was held, entitled: Hormones and Endocrine Disrupters in Food and Water: Possible Impact on Human Health. The workshop was organised by Professor Niels Skakkebaek, the Danish researcher who published a meta-analysis of human sperm quality data in the early nineties. This study, together with the book entitled “Our Stole Future” written by WWF-employee Theo Colborn, stirred up the discussion about endocrine disruption and the possible connection between wildlife effects and human effects. Consequently, substantial research funds were allocated to the subject, especially in the USA and Japan, which triggered a wealth of research on the subject.

The present workshop gave a state-of-the-art overview mainly on human health issues, with some attention for wildlife effects. The general picture emerges of specific wildlife effects at locations of high chemical spills, whereas in terms of human health the situation is less clear. A series of human endocrine-related health conditions have been implicated in the endocrine disrupter debate, but no causal relationship between exposure and health effects has been established sofar. There is a particular lack of actual human exposure data that would enable the study of possible causality. The workshop gave some limited exposure data, which may be a starting point for future analyses of human risk.

A substantial number of outstanding scientists working in this field gave lectures at the workshop, providing a good overview of the current situation in human endocrine disrupter research. In the detailed report given below the outline of the program is followed and comments are given where appropriate.

2. Introductory Lectures

Three introductory lectures from three different perspectives of the subject clearly brought the issue into focus. McLachlan gave an overview of diethylstilbestrol (DES)-related research. DES is a synthetic estrogen, which was used as a drug to prevent spontaneous abortion, and was later found to produce malformations in the genital organs of female and male offspring. In addition, the risk of cervical cancer appeared to be increased in prenatal exposed women. DES has been put forward as the prototype endocrine disrupter. It should be realised, however, that the situation with DES is unique in several ways. There was intentional exposure to high therapeutic doses during pregnancy, and DES has a higher affinity for the estrogen receptor than natural estrogens. Environmental estrogens probably do not reach such high concentrations in the body, and furthermore their estrogenicity is many orders of magnitude lower than natural estrogens.

Ashby has performed a series of studies on endocrine disrupter effects in animal experiments. He alluded to inconsistencies in results between laboratories, which could in one case be explained by differences in feed composition, and in another by differences in exposure route. Furthermore, there is no evidence to date to suggest that endocrine disrupters have synergistic effects. Main issues in this area concern the specificity for the reproductive organs and the possibility that effects may occur at dosages below the established NOAEL, as current testing procedures may not include all relevant endocrine end points. The public interest in the issue has caused any study, which shows effects to get much attention, in spite of the lack of reproducibility of some of the results. Ashby argued for strong scientific scrutiny outside the public arena. In addition he advocated discussions of the relevance of some of the studies, e.g. in vitro estrogenicity assays and in vivo tests where very high effective dosages were used.

Skakkebaek summarised old and new studies on sperm quality and genital malformations in man. New studies have again shown low sperm numbers in young Danish men, which may have consequences for fertility. The differences between ethnic populations, the variability with season, and methodological issues were also touched upon. He suggested the Testicular Dysgenesis Syndrome as a term combining all morphologic and functional end points, suggesting a common cause in adverse environmental influences. However, he did provide neither data to show the common pathogenesis of the various effects noted, nor was any evidence shown for causality in relation to environmental exposures.

Taken together, the introductory lectures clearly illustrated the present state of affairs, which can be summarised as follows. On the one hand we know about the devastating effects of DES exposure in pregnancy, which has made clear that exposure to endocrine compounds may profoundly affect the reproductive system in man. However, as explained above, DES is a very special case, very different from the environmental exposures that we encounter. On the other hand, we see reproductive

anomalies in man, sperm quality studies probably being the most frequently quoted. The missing link is the evidence for a causal relationship with a causative factor such as environmental exposures. Many experimental animal studies have been published or are underway, but their reproducibility and relevance for the actual human situation are subject to doubt. Thus, evidence for a cause and effect relationship regarding human endocrine disruption is still lacking.

3. Trends in Male Reproductive Disorders

Moller reviewed the trends in the incidences of testis and prostate cancer using Danish Cancer Registry data. He showed that for both diseases the increases in incidence have been levelling off, up to even a reduction for prostate cancer. He found no grounds to suggest a strong role for exogenous hormonal exposures in these cancers. Toppari reviewed the incidences in cryptorchidism and hypospadias using data from the International Clearing House for Birth Defects Monitoring Systems. He considered these data as unreliable and noted much possible confounding factors, such as maternal body weight, order of birth, birth weight, prematurely and threatened abortion. Furthermore, he stated that the human fetus is "bathed in estrogen" , due to the absence of estrogen binding proteins, whereas androgens are absent in the fetus, and do not occur before puberty after LH-stimulation. The influence of exogenous estrogenic and androgenic compounds is far from clear. Many possible forms of bias and methodological differences do not allow clear conclusions on causality. Jouannet reviewed trends in semen quality worldwide. He listed a series of cofounders of sperm numbers: ethnic origin, age, sexual activity, medication, stress, diet, clothing, smoking, season, abstinence time, analytical methods and statistics. Iwamoto described the seasonally in sperm concentration, varying between 50 and 90 million per ml on average between the seasons, with highs in autumn and lows in spring. Wang reviewed world-wide trends in male reproductive health and concluded that no adverse trends were visible. She concluded that in view of the relatively high levels of natural estrogens in the human body, other factors than endocrine disrupters may be important in the pathogenesis of reproductive anomalies.

Mori described lifetime changes in testis weight in man. After an increase in youth, peak weight is reached followed by a decrease in later life. An extensive study showed that during the last three decades the weight curve has shifted toward younger age. The causes for this are unclear, they coincide with other studies showing a trend toward earlier puberty. Interestingly, analyses of umbilical cord blood showed that fytoestrogens diadzein, genistein and equol were present in the ppb range, cadmium, lead, bisphenol A and nonylphenol in the ppt range, and DDE, PCB and dioxins in even lower concentrations. At these concentrations it seems unlikely that these compounds have any measurable endocrine effect.

This session showed that little progress has been made in identifying whether endocrine disrupters actually influence male reproductive health. The exposure data shown by Mori showed that actual exposure to endocrine disrupters was very low in his study, most likely far below concentrations that may have an effect on reproductive organ function.

4. Epidemiology of breast cancer: an environmental desease?

Sasco reviewed the extensive literature on breast cancer determinants, including lifetime estrogen exposure and lifestyle factors. No evidence to date exists for a causal role for environmental estrogens in breast cancer. Huff stipulated that the causes for the overwhelming majority of breast and other cancers remain unknown. He proposed that cancer risk is a function of predisposition, environment and age, with environment being the only factor that can be influenced by lifestyle. The possible influence of environmental estrogens was suggested, but not substantiated by data. Russo presented effects of estrogenic compounds on the transformation of cells in culture. Such mechanistic studies may unravel mechanisms of the initiation of breast cancer. This session gave no clues as regards the relationship between breast cancer and endocrine disrupter exposures.

5. Disorders of puberty and juvenile obesity

Sultan and Juul presented the roles of androgens and estrogens respectively on growth and sexual maturation. Bourguignon stated that puberty onset may be prenatally programmed by excess androgens or by removal of negative feedback of sex hormone action. A possible role of endocrine disrupters was suggested. In serum of foreign adopted kids in Belgium 1-10 ng of pp-DDE was found, which was hypothesised to mediate early puberty onset. However, diet and light (?) were also mentioned as possible causative factors. A comment from the adience (Gray) was that pp-DDE is not estrogenic, in contrast to op-DDE. Another comment (Sharpe) suggested that prenatal nutritional programming may influence puberty onset. Partsch described the epidemiology of precocious puberty. No time-trends were noted. Lee (for the USA) and de Muinck Keizer-Schrama (for Europe) showed secular trends toward a lower age at onset of puberty and menarche in western populations, whereas age at puberty seems to stabilise during the last decade. Possible causes mentioned were socio-economic status, health service, urbanisation, nutrition and environmental chemicals. In the discussion that followed, also family conflict, opening up of society, sexual abuse and vegetarianism were mentioned as factors possibly related to early menarche. Anabolic steroid use in cattle for meat production was mentioned as a possible source of human exposure to sex steroids, which was questioned by remarks about the absence of increased mammae growth in boys, and the fact that accidental intake of contraceptives by children has never given rise to observed long-term effects.

Sorensen described the global epidemic of obesity, with its first wave in the birth cohort after WWII and the second wave in the birth cohort of the early sixties. Family studies have shown that genetic predisposition plays a role, however in view of the development of the epidemic environmental factors are probably crucial. Inactivity and food consumption are increased in obesity, but whether these are the causal factors remains unclear. The role of leptin, although its physiological function is unravelled, in the epidemic is still controversial. The relation between birth weight and obesity risk suggests that prenatal programming may play a role, but the exact explanation remains unclear. Surprisingly, since the nineties the prevalence of obesity in young men is rapidly declining, further substantiating the possible role of environmental factors.

In summary, this session showed substantial changes in puberty onset, age at menarche and obesity in western society, suggesting environmental influences. Whether endocrine disruption plays a role in these phenomena is as yet unclear.

6. Effects of hormone disrupters in man and wildlife: Accidents and case studies

Guilette described wildlife effects of environmental endocrine disrupters, e.g. pp-DDE, the main contaminant in lake Apopka in Florida where genital organ malformations were observed in alligators. Matthiessen described endocrine disrupters in English surface water, where 90% of the estrogenic activity was caused by the natural hormone estradiol-17β. Sediments have been shown to contain 5.5 μg E2 equivalent per gram sediment. This was mainly caused by an unidentified nonsteroid nonalkylphenolic compound. Olea described a method for determination of the estrogenic load of human fat tissue. Chiumello described gynecomastia in children upon exposure to estrogenic pharmaceuticals and Swan reviewed the human health effects of DES. Kogevinas reviewed the human toxicology of dioxin and Budstos-Obregon described the adverse human health effects of organophosphorus pesticides. Boersma described subtle neurological effects in a group of 418 children related to prenatal and lactational exposure to PCB. Measurements showed 2 μg/l PCB in maternal blood and 0.5 μg/l PCB in cord blood. Furthermore, at age 3.5 years breastfed babies had 0.7 μg/l PCB in blood whereas formula-fed infants had 0.2 μg/l PCB in blood. Breastfeeding remains preferable for various reasons including better motor development in these children and reduced maternal risk for breast cancer. A large CDC study in 1200 kids has shown no relationship between PCB exposure and child development.

All current studies showing reproductive effects of endocrine compounds in wildlife as well as man relate to high exposures and/or compounds with high estrogenic potency. A series of constraints of current endocrine disrupter research were noted as follows. As regards the evaluation of effects there are multiple endpoints, effects may be non-specific and on the borderline between normal and pathologic, the developmental stage at exposure plays an important role, effects may or may not have a long latency, and susceptibility may vary within and among species. As regards exposure assessment, in reality exposure is always to a mixture of compounds, which in addition is largely unidentified, may occur from various sources such as drugs and contaminants, time and duration of exposure may vary, and methodological deficits may preclude proper exposure assessment. Human actual exposure assessment is currently one of the crucial white spots in endocrine disrupter risk assessment.

7. Toxicology and mechanisms of action of endocrine disrupters in animal models

Newbold gave an overview of experimental research on DES, and stipulated the unique high estrogenicity of the compound. She noted that in view of differences between species in terms of developmental timing, exposure in the mouse should cover the first postnatal week to mimick human prenatal exposure. Cervical adenocarcinoma, one of the typical effects of DES, is not brought about in the mouse by compounds such as bisphenol A, nonylphenol and methoxychlor, which are prominent suspect estrogenic endocrine disrupters.

Foster reviewed the male developmental effects of phthalates, and mentioned the Sertoli cell as the main target for this class of compounds. He showed that gestation day 12 to 21 represents the sensitive period in the rat, which programs the testicular response in adulthood. This exposure period is not covered effectively in the classic developmental toxicity study with exposure between gestation days 6 and 15. New protocols extend exposure to gestation day 21, which prove to be more sensitive in terms of male reproductive toxicity of phthalates. He advocated for additional end points for studying endocrine disruption, such as anogenital distance at birth, nipple retention at postnatal day 13, prenuptial separation at day 40, and adult testis and epididymal pathology.

Vom Saal discussed the possibility that endocrine compounds may not have the classical sigmoid effect curve but instead display a biphasic response with an unexpected response at very low doses. He presented very low dose effects of 2 μg/kg/day of bisphenol A on sperm and prostate in mice, but the reproducibility of these results were challenged by the audience. Spearow showed that endocrine effects may vary as much as four orders of magnitude between parental strains and mixed F1's, indicating that the genetic background of the model system of choice may have an enormous impact on the outcome. Gray reviewed antiandrogens and their effects in experimental animals. Most compounds were effective only at relatively high doses of around 100 mg/kg/day. He showed additivity but no synergism between procymidone and dibutylphthalate with respect to the induction of hypospadias. Veeramachaneni exposed rabbits prenatally to drinking water containing a mixture of commonly occurring environmental chemicals and found subnormal reproductive parameters in adulthood. Brock presented chromatographic methods for detection of phthalates.

This session contained a variety of animal model research showing that endocrine active compounds may cause reproductive toxicity. Therefore adequate risk assessment of such compounds is needed, and the inclusion of additional end points for endocrine effects may be warranted. Especially the relatively high effective doses and the controversial low dose effects need to be weighed against the actual human exposure levels.

8. Hormones in food – their detection and biomarkers of exposure

The use of anabolic hormones for stimulation of growth of cattle for meat production was discussed. Meyer reviewed the effectiveness of these treatments, and Henricks and Maume showed results of determinations of hormone concentrations in meat products. Stephany elaborated on the differences between Europe and the USA. Whereas in Europe hormone use is banned completely, the US allows the use of a restricted number of compounds under specific conditions. Daxenberger showed that meat and milk naturally contains measurable levels of estradiol and derivatives. It is not clear whether additional hormone treatments in meat production provide an additional risk of endocrine disruption.

Larsen presented a Danish food surveillance study in which a.o. exposure to a series of compounds with alleged endocrine disrupting properties was estimated. It appeared that for most compounds the actual exposure was at least one order of magnitude below the ADI. A smaller margin was found for PCB. It was mentioned that in Sweden the actual exposure to TCDD via food was around the ADI. In Denmark, exposure to total phthalates was below the individual TDI's. Of 20 pesticides, among which 4 were estrogenic in vitro, the intake was found to be lower than 1% of the TDI. Such data will be instrumental in human actual risk assessment of endocrine acting compounds.

9. Target pathways and mechanism of action of hormones

Korach decribed research using estrogen receptor knockout mice to study the role of alpha and beta-receptor types in endocrine disruption. Sharpe reviewed an elegant series of experiments showing that the balance between estrogenic and androgenic activity determines the effect. He showed that the effects of DES could be completely abolished by coadministration of testosterone. He further argued that as high doses of DES were necessary to elicit any effect, weaker estrogenic compounds are not likely to reach effective doses in actuality. He also showed that a combination of bisphenol A, octylphenol and 0.01 μg DES accelerated the onset of spermatogenesis and resulted in more sperm cells per sertoli cell. Sheehan argued that for endocrine effects there is no threshold as is assumed in general for reproductive effects. This relates to new insights in reproductive toxicology, where it is realised that the threshold is not a physiological entity but rather a product of study design.

The endocrine disrupter issue has resulted in a wealth of mechanistic research into endocrine mechanisms. The workshop had a series of posters on mechanistic aspects. Their meaning in terms of human risk of endocrine exposure and effects is difficult to estimate.

10. Conclusions

Various human endocrine related diseases have given reason for concern regarding exposure to endocrine acting xenobiotic compounds. Increasing incidences of breast and testicular cancer and variations in sperm quality have been noted. The connection of these findings to exposure to endocrine acting xenobiotics seems obvious from a mechanistic point of view, but a causal relationship is hard to prove. This workshop has shown that we are still uncertain about the existence of endocrine disruption in man, apart from the specific case of DES. However, new data are emerging about the actual exposure of man to alleged endocrine disrupters. Sofar, observed exposures were well below doses at which endocrine effects are to be expected. More exposure data are needed to found a solid basis for actual human risk assessment in the field of endocrine disruption.