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Comparative assessment of endocrine modulators with oestrogenic activity: I. Definition of a hygiene-based margin of safety (HBMOS) for xeno-oestrogens against the background of European developments

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Abstract A novel concept – the hygiene-based margin of safety (HBMOS) – is suggested for the assessment of the impact of potential endocrine modulators. It integrates exposure scenarios and potency data for industrial chemicals and naturally occurring dietary compounds with oestrogenic activity. An HBMOS is defined as a quotient of estimated daily intakes weighted by the relative in vivo potencies of these compounds. The Existing Chemicals Programme of the European Union provides Human and Environmental Risk Assessments of Existing Chemicals which include human exposure scenarios. Such exposure scenarios, along with potency estimates for endocrine activities, may provide a basis for a quantitative comparison of the potential endocrine-modulating effects of industrial chemicals with endocrine modulators as natural constituents of human diet. Natural phyto-oestrogens exhibit oestrogenic activity in vitro and in vivo. Important phyto-oestrogens for humans are isoflavones (daidzein, genistein) and lignans, with the highest quantities found in soybeans and flaxseed, respectively. Daily isoflavone exposures calculated for infants on soy-based formulae were in the ranges of 4.5–8 μg/kg body wt.; estimates for adults range up to 1 mg/kg body wt. The Senate Commission on the Evaluation of Food Safety (SKLM) of the Deutsche Forschungsgemeinschaft has also indicated a wide range of dietary exposures. For matters of risk assessment, the SKLM has based recommendations on dietary exposure scenarios, implying a daily intake of phyto-oestrogens in the order of 1 mg/kg body wt. On the basis of information compiled within the Existing Chemicals Programme of the EU, it appears that a daily human exposure to nonylphenol of 2 μg/kg body wt. may be a worst-case assumption, but which is based on valid scenarios. The intake of octylphenol is much lower, due to a different use pattern and applications, and may be neglected. Data from migration studies led to estimations of the daily human uptake of bisphenol A of maximally 1 μg/kg body wt. On the basis of comparative data from uterotrophic assays in rats, with three consecutive days of oral applications involved, and taking the natural phyto-oestrogen daidzein as reference (= 1), relative uterotrophic activities in DA/Han rats follow the sequence: daidzein = 1; bisphenol A = 1; p-tert-octylphenol = 2; α, p′-DDT = 4; ethinyl oestradiol = 40,000. The derived values from exposure scenarios, as well as these relative potency values and bridging assumptions, led to calculations of HBMOS as a quantitative comparison of potential endocrine-modulating effects of industrial chemicals with those of natural constituents of human diet. HBMOS estimates for nonylphenol ranged between 250 and 500, dependent on bridging assumptions, and around 1000 for bisphenol A. The derivations of HBMOS were in full support of the conclusions reached by the SKLM of the Deutsche Forschungsgemeinschaft. The estimated HBMOS values for the industrial chemicals (nonylphenol, bisphenol A) appear sufficiently high to ensure the absence of a practical risk to human health under the present exposure conditions.

Key words Endocrine modulators · Hormonally active agents · Endocrine disruptors · Nonylphenol · p-tert-Octylphenol · Bisphenol A · α, p′-DDT · Daidzein · Genistein · HBMOS · Hygiene-based margin of safety

Introduction

The term “endocrine-disrupting chemicals” for compounds with an effect on the endocrine system was
introduced by Colburn et al. (1993). Subsequently, this
denomination has been introduced into official use, in
the United States (for review see Crisp et al. 1998), in
the European Union (1999) and in Japan. As an alternative,
the more neutral term “endocrine modulators” is also
used within the scientific community (DGPT 1999; Bolt
and Degen 2000; Nilsson 2000; Fenner-Crisp 2000). In
the United States, the National Research Council (1999)
has recently issued a report of its Committee on
Hormonally Active Agents in the Environment; it was
explicitly stated that some Committee members had
objected to the terms “hormone disruptor” or “endo-
crine disruptor” as pejorative, and it was decided to use
the term “hormonally active agents (HAA)” to describe
these compounds. In view of this discussion, the present
publication preferentially adheres to the term “en-
docrine modulator(s)”, as used by the Toxicology Advisory
Committee of the German Society of Experimental and
Clinical Pharmacology and Toxicology (DGPT 1999).

As also pointed out by the DGPT (1999), the term
“xeno-oestrogen”, by analogy with the usual term “xe-
nobiotics”, should be defined as a compound with oes-
trogenic activity that is not produced in the organism,
but is found in the environment. Thus, the definition of a
“xeno-oestrogen” covers both man-made oestrogenic
chemicals and those of plant origin (phyto-oestrogens);
it closely resembles the term “environmental oestrogens”
(McLachlan et al. 1984; Mäkelä et al. 1999). This is not
merely a matter of semantics: The use of the term “xeno-
oestrogen” exclusively for man-made chemicals with
oestrogenic properties, in contrast to naturally occurring
oestrogenic compounds (phyto-oestrogens), suggests a
distinction (dichotomy) to be made by the public.

As far as the official discussion in the European Union
is concerned, a first European Workshop on the Impact
of Endocrine Disrupters on Human Health and Wildlife
was convened in 1996 in Weybridge, UK, to discuss funda-
mental issues in this field. It was agreed that an endocrine
disrupter (disruptor) could be adequately defined only in
terms of adverse effects on intact animals, although
identification of potential endocrine disrupters was
deemed possible using in vitro systems, and definitions of
“endocrine disrupters” and “potential endocrine dis-
rupters” were endorsed. The report was published by the
Environment and Climate Research Programme (DG

In late 1999, the European Commission adopted a
document Community Strategy for Endocrine Disruptors,
intended as a “non-binding communication” proposing
actions within the framework of the Existing Chemicals
management policy. In general, “endocrine disruption”
was viewed as a “mechanism whose effects relate to the
functions of the endocrine system”. It was also stated
that two classes of compounds could cause endocrine
disruption, namely natural hormones (body-specific
hormones and phyto-oestrogens) and man-made chemi-
cals (designed for use in industry, in consumer goods
and as by-products). The communication of the Euro-
pean Commission (1999) also mentions the legal and
legislative instruments which might be used to take
regulatory measures.

Furthermore, the communication of the European
Commission (1999), on the basis of results of both the
Weybridge conference (see above) and a subsequent
joint meeting of the ICPS (International Programme on
Chemical Safety of the World Health Organization) and
the OECD (Organization for Economic Cooperation
and Development) (Anonymous 1998), endorses the
following “European definitions”:

- “An endocrine disruptor is an exogenous substance
or mixture that alters function(s) of the endocrine
system and consequently causes adverse health
effects in an intact organism or its progeny, or
(sub)populations.

- A potential endocrine disruptor is an exogenous sub-
stance or mixture that possesses properties that might
lead to endocrine disruption in an intact organism
or its progeny, or (sub)populations”.

The Commission recognizes that “the main case
studies concerning the phenomenon of endocrine dis-
ruption associate adverse effects with exposure to high
levels of specific chemical substances”. This, and the fact
that the Commission intends to address the problem
within the Existing Chemicals regulation framework,
may be viewed as an indication that it is accepting the
established threshold levels and safety factors concept,
as identified in toxicology.

Another potential key element for a further scientific
discourse is the recognition (see above) that not only
man-made chemicals must be considered, but also hor-
mones of natural origin, i.e., body-specific hormones
and phyto-oestrogens. In 1995, Safe has put forward the
idea of identifying environmental/dietary oestrogens and
antioestrogens and of estimating “oestrogen equiva-
Ients” to compare the relative dietary impacts of various
classes of oestrogenic chemicals. Specifically, he pointed
out that risk assessment of xeno-oestrogens and other
synthetic chemicals which modulate endocrine responses
must take into account the high dietary levels of natural
compounds in food, and also in drugs and herbal
extracts sold in health food stores.

Making use of exposure scenarios provided within
the Existing Chemicals programme of the European
Union, we have published a preliminary assessment
comparing the relative potentials for endocrine modu-
lation of an industrial chemical, nonylphenol, and a
natural phyto-oestrogen, daidzein (Bolt et al. 1998). The
present communication proceeds further along this
avenue, using the more complete data which became
available only very recently.

**Techniques for the detection of oestrogenicity**

An understanding of the molecular mechanisms
involved in hormone action in general, and specifically