As *in vivo* screening tests, the uterotropic (for (anti-)estrogenicity) and the Hershberger assay (for (anti-)androgenicity) have both been mentioned by the US EPA and the OECD. Further proposals of the EPA are for a 20-day pubertal female assay or a 14-day intact adult male assay, which also include testing for thyroid effects.

Definitive *in vivo* mammalian testing is focused on the two-generation test or another long-term procedure covering the whole reproductive cycle, including intrauterine development. In addition, the OECD is proposing classical subacute or subchronic tests for identification and possibly characterization of EACs. It may become necessary to enhance the existing test guidelines by additional hormone-specific parameters. This was proposed in the *Detailed Review Paper: Appraisal of Test Methods for Sex-Hormone Disrupting Chemicals,* prepared by UK authorities for the OECD (QEOD, 1997).

When looking at these proposals in detail, it becomes obvious that the suggested test methods have not been sufficiently validated. Thus, as a prerequisite for laying down definite test guidelines for regulatory purposes, a systemic validation effort is necessary. This regards both the relevance as well as the reliability.

The relevance of a test method is defined by its end point in relation to the different mechanisms by which chemicals may interact with the endocrine system, as follows: direct (cyto)toxicity on endocrine active or responsive tissues; interference with biosynthesis, metabolism or excretion/clearance of hormones; and interaction with hormone receptors, either as agonists or antagonists

Obviously, the relevance of each proposed test method may be different, as in the examples given below.

In vitro receptor-binding/transcriptional activation assays only address direct hormone receptor interactions. (Cyto)toxic effects or alterations in hormone biosynthesis for metabolism are not taken into account.

Two *in vitro* assays for hormone biosynthesis are under discussion in the EPA scheme, the "steroidogenesis assay with minced testes" and the placental aromatase assay". Both these assays only cover a small segment of the steroid hormone biosynthesis pathways and they do not give any indication at all of the metabolic deactivation of steroids. On the other hand, tests in the intact animal should, in principle, show any effect on the endocrine system regardless of the occurring mechanism, but offen the mode of action may remain unclear.

As regards the reliability of the test methods to be established, a worldwide validation process has now been initiated by the OECD for the uterotropic and the Hershberger assay as well as for the subacute 28-day test (OECD test guideline 407) enhanced by additional hormone-specific parameters. Laboratories used to routine testing for regulatory purposes, such as those from industry, must be predominantly involved in such a validation process. They must be capable of simultaneously handling a large number of animals and they must have a broad expertise in the clinical examination of animals, histopathology, clinical chemistry and hematology.

In the overall validation process of test methods for regulatory purposes of EACs, the following points are of utmost importance for the chemical industry:

i) The overall test strategy and each single test procedure should be accepted worldwide.
ii) Test methods must be specifically designed to a clearly defined purpose; there must be a differentiation between screening for effects, hazard identification, risk assessment purposes and finally mechanistic research tools.
ii) The test methods must have a sound scientific basis, they must be robust and yield reproducible results.
iv) The test methods should be "slim"; unnecessary parameters should be avoided, even if they interesting from a scientific point of view.
v) Costs and practicability of the tests should be an important, but of course not the decisive consideration, especially for screening tests and those to be used for routine investigations.
vi) The test guidelines should be broadly validated in an international effort to become accepted by all regulatory authorities worldwide.
vii) Validation of each test method must cover sensitivity, specificity and reproducibility.
viii) For the validation process, appropriate reference chemicals must be identifled and the same test materials should be used worldwide.

In summary, taking account of the uncertainties with regard to the possible impact of EACs on the environment or human health, it is necessary to establish screening and testing procedures for hazard identification and risk assessment purposes. Such tests should ideally cover all possible modes of action of EACs. Numerous regulatory authorities and organizations worldwide take part in this process, *e.g.,* the US EPA and the OECD. A critical review of the proposed test strategies or single test methods reveals they all lack sufficient validation. Before any test guideline can be finalized for regulatory purposes, an in-depth investigation of its relevance and reliability is necessary. Due to their specific expertise in handling large numbers of animals and measuring a wide variety of parameters simultaneously, industry laboratories must take an active part in this worldwide validation process.

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Pulmonary toxicity and risk assessment of pesticides contained in house dust and smoke

J. Pauluhn

Bayer AG, Institute of Toxicology Wuppertal, Germany

Pesticides in general and pyrethroids in particular have received considerable attention as they are widely used for numerous applications, ranging from food protection to general pest control in the indoor environment. The pyrethroids have been proven useful in the domestic environment as room and surface sprays, in passive and active evaporator systems, in incense products such as mosquito coils or to make textiles insect resistant. The specific physicochemical properties of most pyrethroids, *i.e.,* low vapor pressure and high lipophilicity, minimize the extent of passive inhalation exposure but favor their adsorption in house dust. Pyrethroids act directly on the axon through interference with the sodium channel

gating mechanism that underlies the generation and conduction of each nerve impulse (1, 2). One notable form of toxicity associated with some pyrethroids, *e.g.,* the type II pyrethroids containing an a-cyano group, has been facial cutaneous paraesthesia (transient sensations) and irritation-related respiratory symptoms mainly observed in highly exposed workers spraying pyrethroids or in occupational settings. The time course of these sensations is usually immediate or within a few minutes after contact (3). Such effects are considered to be the most prominent health symptoms known to accompany direct contact with this class of pesticide. Based on this rationale, it seems appropriate to utilize animal models to address this physiological end point and to analyze whether pyrethroids act as agents known to elicit upper respiratory tract sensory irritation (4) and whether this mode of action is changed when they are associated with particles.

Interest in indoor air quality is steadily increasing. The factors which affect indoor air quality and the health effects reportedly associated with it are the subject of intense debate. One of the issues which generates the most interest and emotion is related to the exposure to pesticides used indoors and their associated health effects. Indoor exposure to pesticides is complex and varies from short-term high-level exposure, as is likely to occur during the use of spray cans, to long-term low-level exposure to effluents of slow-release devices which are commonly used overnight, for example, mosquito coils or vaporiser systems (5, 6). Particularly for the latter group of indoor insecticides, the actual exposure pattern of humans is difficult to assess because the concentrations of airborne particulates containing pesticides vary with time, room ventilation, and proximity to the source. Since not all of their components are removed or translocated from the indoor environment at the same rate, the concentrations of volatile and nonvolatile components also vary in relation to each other over time. In addition, confounding factors attributing to the overall particle load, such as environmental tobacco smoke, have to be considered. These aspects demonstrate that well standardized sampling strategies are required for analytical determinations and differentiation of actual or potential sources of human exposure.

Due to the complexity of the pattern of indoor exposure to pesticides, sampling strategies differ considerably, ranging from determination of pesticides in sedimented house dust to measurements in airborne dust or body fluids. Because of the very low vapor pressure of most pyrethroids, their concentration in indoor air was found to be very low and passive and long-term exposure of adult humans is commonly thought to occur via resuspended contaminated house dust. This assumption has promoted the development of a number of sampling strategies for the determination and assessment of the external and internal dose of such pesticides. Approaches for the determination of the external dose ranged from taking dust from contaminated surfaces by wipe sampling, by analysis of house dust from vacuum cleaner bags or by taking samples from indoor air. Each medium may favor a specific route of exposure, *i.e.,* oral uptake by toddlers or small children through swallowing contaminated dust, licking hands or toys, direct dermal uptake via contaminated surfaces or inhalation uptake via airborne dust, smoke or aerosol.

The potential health risk arising from such complex exposure scenarios can only be evaluated by inhalation toxicity studies of adequate duration with the active ingredient, individual carrier substances likely to become airborne or even of the entire mixture. In this instance, hazard identification and risk assessment of smoke released from mosquito coils appears to be most complex because of the combustion of organic material from which the mosquito coil is made. Thus, a great number of ingredients are subject to evaporation and/or combustion, *e.g.,* sawdust, coconut shell powder, starch, binders, fungicides, insecticides, synergists and other additives that may, under certain circumstances, cause and/or exacerbate specific portal-of-entry effects which cannot be studied and assessed by noninhalation routes of exposure. Thus, the toxicological assessment of mosquito coil smoke atmospheres appears to be most challenging since the shuttle function of smoke particulates may facilitate the penetration of specific agents into the lower respiratory tract that would normally have been deposited in the upper respiratory tract. Thus, for mosquito coil smoke, the location of the major deposition of specific constituents contained in the smoke mixture may be contingent upon their behavior in the smoke mixture. Irritant combustion gases and an accumulation of particles are likely to affect the detoxification pathways of the respiratory tract which differ from location to location, *i.e.,* nasal cavities, larynx, trachea, bronchial airways, and alveolar region. This, in turn, may interfere with the pathomechanism(s) of the active ingredient and agents likely to accumulate in highly specialized cell populations present only in the alveolar region.

The objective of the studies presented is to analyze the suitability of various exposure regimens to evaluate which bioassay provides the relevant data for risk assessment for which household insecticide. Particular emphasis is made to test the most complex entity, namely mosquito coil smoke atmospheres, to characterize whether the acute sensory irritation inhalation rodent bioassay, a subacute or a subchronic inhalation study or even more in-depth approaches are required to allow evaluation and assessment of their toxic potency. Due to the complex nature of exposure atmospheres generated by mosquito coils, it is scientifically challenging to characterize the pathomechanism of most concern, since irritant combustion gases, volatile and semivolatile organic substances, particulates (soot), condensation aerosols and active substances recondensed onto particulates may act independently, synergistically or mixture specifically. Despite the presence of mild respiratory tract irritation, mosquito coil smoke did not demonstrate adverse effects caused by pyrethroid(s) or smoke particles, The histopathological effects elicited with mosquito coil smoke were confined to irritant effects in the upper respiratory tract (nasal cavities) which appeared to be causally related to wood combustion products, such as formaldehyde or acrolein, and are quite comparable to those obtained with environmental tobacco smoke. The data generated with mosquito coil smoke appear to suggest that the use of mosquito coils is not accompanied with any undue risk, although it has been shown that combustion effluents originating from the coil matrix may elicit upper and lower respiratory tract irritation.

Passive exposure to household insecticides may also raise concerns as nonvolatile active ingredients may be adsorbed by house dust. Many analytical surveys addressing indoor contamination have demonstrated appreciable amounts of pesticides bound to house dust and concerns have been raised as to whether this contamination is of any toxicological relevance. Accordingly, for the assessment of pyrethroid contamination of house dust, elaborate inhalation studies were conducted using "sprayed" or "dusted" carpets. The objective of these studies was to address the question as to whether pesticide contamination in sedimented house dust can serve as an indicator to predict potential inhalation exposure. Experimental evidence as well as human biomonitoring studies sug-

gest that, following treatment of carpet, only a very small fraction of the applied pyrethroid can indeed be regarded as potentially bioavailable by inhalation (max. 0.2% of the active ingredient applied to the carpet). Therefore, assessment of health hazards in the indoor environment based solely on "vacuum cleaner" sampling (as used by analytical chemists to search for indoor contaminants) rather than examination of the actual airborne concentration, including other relevant airborne materials, is prone to tremendous errors and misjudgments.

These examples demonstrate that new regulatory requirements, *e.g.,* the EU biocide directive, and public perception and concern may trigger complex and demanding toxicological examinations and standardization of such complex studies with regard to the generation of test atmospheres, mode and duration of exposure, and selection of adequate toxicological endpoints, as a basic prerequisite for state-of-the-art hazard identification and risk assessment.

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Adverse effects of environmental exposures may occur not only in the individuals directly exposed, but also in their progeny

L. Tomatis[1] and U. Mohr[2]

[1]Cave 25/r, Aurisina (Trieste), Italy, and [2]Institute of Experimental Pathology, Hannover Medical School, Hannover, Germany

Individual differences in the risk of cancer are ultimately determined by the interaction between the environment, that is the complex of factors that are not part of the genetic patrimony at the time of conception, and the genotype. Beginning prenatally, humans are exposed throughout life to a variety of environmental agents, among them many carcinogens and mutagens. Adverse effects of such exposure may occur not only in the individuals directly exposed, but also in their progeny.

Prenatal events may contribute to increase the cancer burden following either: i) exposure to a noxious agent during pregnancy with consequent direct exposure of fetal cells *in utero* (transplacental carcinogenesis), or ii) exposure of one or both parents to a carcinogen/mutagen before conception, resulting in a possible alteration of the germ cells (transgenerational carcinogenesis).

There is experimental evidence of a transplacental carcinogenic effect for a large number and variety of chemical carcinogens. Evidence in humans is instead limited to exposure during pregnancy to diethyisfilbestrol (DES) and X-rays. Studies in rodents, however, cover the entire lifespan of the progeny and the increase in cancer frequency is generally observed in adult or late life. Observations in humans are almost exclusively confined to the occurrence of Cancer in childhood.

Even if many experimental studies remain open to criticism, particularly due to the relatively small number of animals used and the consequent lack of statistical power of the data, there is convincing evidence for a transgenerational effect in laboratory animals of a few chemical Carcinogens and of internal and external exposure to radiation. The epidemiological data in humans are mostly derived from the investigation of paternal occupational exposures to chemicals, chemical mixtures or radiation and, although suggestive of a transgenerational effect, they remain Controversial. Observations in humans, again, are limited to the occurrence of childhood cancer.

The hypothesis has been proposed that certain environmental exposures of parents before conception may cause alterations of the germ cells which affect the susceptibility of the progeny to cancer. Most likely, different mechanisms underlie the high incidence of tumors appearing at an early age in certain familial syndromes, and the surfacing of an increased predisposition to cancer revealed by a relatively modest increase in tumor incidence late in life. A better understanding of pre- and postnatal gene-environment interactions in determining cancer risks would have important implications for public health, as it could considerably improve the efficacy of primary prevention.

Experimental adenocarcinoma of the esophagus: Implications in the sequence Barrett's esophagus-adenocarcinoma

M. Pera

Hospital Clínic i Provincial de Barcelona, Spain.

No suitable models of esophageal carcinogenesis were available until the discovery by Duckrey *et al.* that N-methyl-N-nitrosoaniline given orally to rats resulted in a high incidence of squamous cell carcinomas of the esophagus. Since then, most of the experimentally induced squamous cell carcinomas of the esophagus have been due to the exposure of rats to several nitrosamines by various routes of administration.

In recent years, the incidence rates for adenocarcinoma of the esophagus have risen rapidly in Western countries, especially among white males. Most of these tumors arise from areas of columnar-lined esophagus (Barrett's esophagus), a condition clearly associated with a chronic gastroesophageal reflux of acid and duodenal-content secretions. These epidemiological observations prompted us