

Maisons-Alfort, (date)

The Director General

OPINION

of the French Agency for Food, Environmental and Occupational Health & Safety

**on a request for scientific and technical support
regarding the European strategy for endocrine disruptors**

ANSES [the French Agency for Food, Environmental and Occupational Health & Safety] undertakes independent and pluralistic scientific expert assessments.

ANSES's public health mission involves ensuring environmental, occupational and food safety as well as assessing the potential health risks they may entail.

It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with all necessary information concerning these risks as well as the requisite expertise and scientific and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its Opinions are made public.

On 6 September 2011, ANSES received a request from the Directorate-General for Food¹ (DGAL) for scientific and technical support regarding the European strategy for endocrine disruptors.

1. BACKGROUND AND PURPOSE OF THE REQUEST

In the framework of EU work related to the European strategy for endocrine disruptors, the European Commission ordered a state-of-the-art report on their assessment. The first section of this report was recently published as an interim report.

This report, as well as the action plan drawn up by the European Commission and proposals by the Member States, were presented on two information days: 26 November 2010 and 19 May 2011.

ANSES was requested to analyse the work currently being undertaken in Europe and at the OECD and the possible consequences of these changes in the system for the assessment of plant protection product authorisations. ANSES was also requested to propose criteria for the definition of endocrine disruption.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with the French standard NFX50-110 "Quality in Expert Appraisal Activities – General Requirements of Competence for Expert Appraisals (May 2003)".

The response to this request for scientific and technical support was prepared by the Regulated Products Department. It was presented to the Expert Committee on 'Plant Production Products: chemical substances and preparations' on 25 October 2011.

¹ of the French Ministry of Agriculture

3. THE AGENCY'S CONCLUSIONS AND RECOMMENDATIONS

First part: Review of work undertaken in Europe and at the OECD

This first section of this Opinion summarises, on the one hand, the main points of the European Commission's action plan, which is based on the European Commission's 4th [interim] report² on the implementation of the European Community's 1999 strategy for endocrine disruptors. Second, the present Opinion then briefly summarises the 2nd Interim Report on the state of knowledge on endocrine disruptors, prepared at the Commission's request by an external consultant.

Every effort has been made to ensure that these summaries are as accurate as possible. Their purpose is to present the current state of European thinking on how endocrine disruptors should be addressed within existing EU regulations, and not to present the Agency's position on the issue of endocrine disruption in terms of human and environmental health.

This first section also presents various proposals that have been made by Member States, non-governmental organisations and industry on the definition of endocrine disruption.

1 BACKGROUND

In order to respond quickly and effectively to concerns regarding endocrine disruptors, the European Commission adopted a Community strategy on endocrine disruptors in December 1999³. This strategy sets out essential requirements for further research, international co-operation, communication to the public and policy action. It recommends short-, medium- and long-term actions. On 30 March 2000, the Environment Council adopted Conclusions on the Community strategy, in which it stressed the precautionary principle, the need to develop quick and effective risk management strategies and the need for consistency with the overall chemicals policy. On 26 October 2000, the European Parliament adopted a Resolution on endocrine disruptors, emphasising the application of the precautionary principle and calling on the Commission to identify substances for immediate action. The Council invited the Commission to report back on the progress of the work at regular intervals, and in December 2009 it invited the Commission to include in its report recommendations as to how exposure to multiple endocrine disruptors should be further addressed within existing Community legislation. To date, the Commission has produced four reports^{4,5,6,7}, the most recent of which is summarised below.

1.1 Progress on short-term actions

1.1.1 List of Priority Substances

The Commission developed a priority list of substances on the basis of their possible endocrine-disrupting properties. The database containing the information used to establish this list was made available on DG Environment's website⁸. The Commission is currently examining the possibility of setting up an interactive web-based platform on substances that have endocrine-disrupting effects.

1.1.2 Communication to the Public

Various websites are used to communicate to the public:

² Commission staff working document on the implementation of the Community strategy for endocrine disruptors. SEC(2011) 1001 final.

³ Communication from the Commission to the Council and the European Parliament-Community Strategy for Endocrine Disruptors. COM (1999) 706 final.

⁴ Communication from the Commission to the Council and the European Parliament on the implementation of the Community Strategy for Endocrine Disruptors. COM (2001) 262 final.

⁵ Commission staff working document on implementation of the Community Strategy for Endocrine Disruptors. SEC (2004) 1372.

⁶ Commission staff working document on the implementation of the Community strategy for endocrine disruptors. SEC (2007) 1635.

⁷ *Op. cit.*, footnote 2.

⁸ http://ec.europa.eu/environment/endocrine/strategy/short_en.htm

- a website devoted to general information and the Commission's activities on endocrine disruptors, managed by DG Environment⁹,
- a website dedicated to research into endocrine disruptors, managed by DG Research (RTD)¹⁰,
- a website managed by the European Commission's Joint Research Centre (DG-JRC)¹¹.

1.1.3 Monitoring Programmes

Many monitoring programmes on chemical substances are carried out at national, European and global levels. However, there is little coordination regarding the way in which data are collected, managed, assessed and reported across the different monitoring programmes. The Commission is currently examining how to address this problem.

1.2 Progress on medium-term actions

1.2.1 Identification and assessment of endocrine disruptors

The Commission and several Member States have initiated work on possible criteria for the identification of endocrine disruptors. The OECD is working on developing guidelines for testing potential endocrine-disrupting effects. Seven test methods have so far been adopted and several others are being developed (see the list of these guidelines in Annex 1).

1.2.2 Research

Several European research programmes have been set up. These include:

- The CREDO cluster (Cluster of Research on Endocrine Disruption in Europe)¹², which demonstrated that the conventional approach for estimating no-observed-effect-levels is inadequate when it comes to taking into account low-dose effects. It has also improved knowledge on the combined effects of several substances with a common mode of action.
- The REPROTECK¹³ project, whose aim was to develop *in vitro* testing methods. More than 20 tests reflecting possible reproductive effects have been set up.
- The CASCADE¹⁴ network of excellence, whose aim has been to remodel the research environment in Europe.
- The NEWGENERIS¹⁵ project, which focuses on the role of exposure to genotoxic substances in the development of childhood cancers and immune disorders.
- The PHIME¹⁶ project, which focuses on the long-term impact of repeated exposure to low doses of several substances in susceptible populations.
- The BIOCOP¹⁷ project, which works on developing new technologies to screen for multiple contaminants in food.
- The NECTAR¹⁸ cluster (Network for Environment Chemical Toxicants Affecting Reproduction), which focuses on the impact of foetal exposure to endocrine-disrupting substances.
- The OBELIX¹⁹ project, which investigates whether prenatal exposure to endocrine disruptors plays a role in the later development of certain disorders (such as obesity).
- The PERFOOD²⁰ project, which focuses on perfluorinated compounds (PFCs) in food.

1.3 Progress on long-term actions

● REACH

Substances identified as having endocrine-disrupting properties may undergo the authorisation procedure in the framework of the European Union REACH²¹ Regulation no. 1907/2006.

⁹ http://ec.europa.eu/environment/endocrine/index_en.htm

¹⁰ http://ec.europa.eu/research/endocrine/index_en.html

¹¹ http://ihcp.jrc.ec.europa.eu/our_activities/cons-prod-nutrition/endocrine_disrupters

¹² http://ec.europa.eu/research/endocrine/projects_clusters_en.html

¹³ <http://www.reprotect.eu/>

¹⁴ <http://www.cascadenet.org/>

¹⁵ <http://www.newgeneris.org/>

¹⁶ <http://www.phime.org/>

¹⁷ <http://www.biocop.org/>

¹⁸ <http://www.nectarcluster.eu/>

¹⁹ <http://www.theobelixproject.org/>

²⁰ <http://perfood.eu/>

Authorisation applies, with no tonnage limits, to Substances of Very High Concern (SVHC) under Article 57 of the REACH Regulation, i.e., substances classified as Carcinogenic, Mutagenic or Toxic for Reproduction, Category 1 or 2; Persistent, Bio-accumulative and Toxic (PBT) substances; very Persistent and very Bio-accumulative (vPvB) substances as defined by Annex XIII of the REACH Regulation; and substances which give rise to a level of concern equivalent to the aforementioned effects, and particularly endocrine disruptors.

Therefore, a manufacturer, importer or downstream user shall not, after a certain date, place that substance on the market for a use, or use it themselves without prior authorisation, unless the use has been exempted. An authorisation may be granted if it has been shown that the risks arising from the use of the substance are adequately controlled or there are no suitable alternative substances or technologies and the socio-economic benefits outweigh the risks.

Article 57(f) of the REACH Regulation more specifically applies to substances having endocrine-disrupting properties, as it states that Substances of Very High Concern include "*substances – such as those having endocrine-disrupting properties [...] for which there is scientific evidence of probable serious effects to human health [...] which give rise to an equivalent level of concern to those of other substances listed in points (a) to (e)*" of the said article, and particularly substances classified as Carcinogenic, Mutagenic or Toxic for Reproduction, category 1A or 1B.

The guidance document that was written for the competent authorities for the preparation of a dossier on the identification of Substances of Very High Concern²² gives a general interpretation of this article that considers that a key part of the definition of a substance with an "*equivalent level of concern*" relates to there being scientific evidence of probable serious effects to humans or the environment. Moreover, it specifies that an additional aspect to be considered is the uncertainty of standard risk assessment for such substances and the health consequences of the risk assessment being erroneous.

- **Plant Protection Products**

Substances identified as having endocrine-disrupting properties that may cause adverse health effects cannot be authorised, in accordance with Regulation (EC) no. 1107/2009²³. Moreover, by 14 December 2013, the Commission is required to present a draft of the measures concerning specific scientific criteria for the determination of endocrine-disrupting properties in relation to human health (Annex II, point 3.6.5 of Regulation (EC) no. 1107/2009).

However, pending the adoption of these criteria, substances that are or have to be classified, pursuant to the provisions of Regulation (EC) no. 1272/2008²⁴, as Carcinogenic Category 2 or Toxic for Reproduction Category 2 shall be considered as having endocrine-disrupting properties.

In addition, substances, such as those that are or have to be classified, pursuant to the provisions of Regulation (EC) no. 1272/2008, as Toxic for Reproduction Category 2 and which have toxic effects on the endocrine organs, may be considered as having such endocrine-disrupting properties.

²¹ Regulation (EC) no. 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) no. 793/93 and Commission Regulation (EC) no. 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC

²² ECHA. (2007). Guidance for the preparation of an Annex XV dossier on the identification of substances of very high concern (p. 58).

²³ Regulation (EC) no. 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC

²⁴ Regulation (EC) no. 1272/2008 of the European Parliament and of the Council of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) no. 1907/2006.

With regard to environmental impacts, active substances having endocrine-disrupting properties that may have adverse effects on non-target organisms also cannot be authorised. However, in contrast to effects on human health, the Commission is not required to present criteria for the determination of these properties in relation to non-target organisms.

Lastly, if a substance is considered as having endocrine-disrupting properties that may cause adverse effects in humans, it shall only be approved if it is considered as a candidate for substitution in accordance with Article 24 of Regulation (EC) no. 1107/2009.

- **Biocides**

The future Biocidal Products Regulation, which will repeal Directive 98/8/EC²⁵, will be very similar to Regulation (EC) no. 1107/2009 with regard to endocrine disruptors and the criteria for approving biocidal substances.

- **Cosmetics**

Substances with endocrine-disrupting effects are not currently restricted under Regulation (EC) 1223/2009²⁶. However, the Commission will be required to review this Regulation with regard to substances with endocrine-disrupting properties, when criteria for identifying endocrine disruptors are available, or at the latest by 11 January 2015.

- **Food Contact Materials**

The Commission, based on the Opinion²⁷ published by EFSA's Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) and applying the precautionary principle, prohibited the use of Bisphenol A in infant feeding bottles. However, it maintained its authorisation for use in other plastic food contact materials with a specific migration limit of 0.6 mg/kg of food.

- **Water**

Directives 2000/60/EC²⁸ and 2008/105/EC²⁹ address the control of chemical substances that pose a risk to the aquatic environment and/or to human health via the aquatic environment; these may include endocrine-disrupting substances and are considered as priority hazardous substances. In autumn 2011, the Commission was due to present a list of priority hazardous substances applying the prioritisation principles outlined in Article 16 of Directive 2000/60/EC.

- **Occupational Safety and Health**

When endocrine disruptors may present a risk to the health and safety of workers, the regulatory requirements in Directive 98/24/EC³⁰ will apply. The Commission shall propose Indicative Occupational Exposure Limit Values (IOELVs) to be applied at EU level.

- **Toys**

Directive 2009/48/EC³¹ improves the existing rules for the marketing of toys that are produced in and imported into the EU, with a view to reducing toy-related accidents and achieving long-term health benefits. In particular, it provides references on substances by limiting the amounts of certain chemicals that may be contained in materials used for toys. These provisions can be applied to endocrine disruptors on a substance-by-substance basis.

²⁵ Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market

²⁶ Regulation (EC) no. 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products

²⁷ Scientific Opinion on Bisphenol A: evaluation of a study investigating its neurodevelopmental toxicity, review of recent scientific literature on its toxicity and advice on the Danish risk assessment of Bisphenol A. EFSA Journal 2010; 8(9): 1829 {116pp}

²⁸ Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy

²⁹ Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC

³⁰ Council Directive 98/24/EC of 7 April 1998 on the protection of the health and safety of workers from the risks related to chemical agents at work (fourteenth individual Directive within the meaning of Article 16(1) of Directive 83/391/EEC)

³¹ Directive 2009/48/EC of the European Parliament and Council of 18 June 2009 on the safety of toys

1.4 Exposure to multiple endocrine disruptors

In response to the request from the Council, the Commission has examined the way that exposure to multiple endocrine disruptors is currently addressed in EU legislation.

The existing EU legislation already offers some possibilities for assessing the cumulative effects of several substances with regard to their effect on the endocrine system. Within the framework of the EU legislation relating to plant protection products, methodologies are being developed to assess the cumulative effect of active substances. These methodologies, once validated, will also be applicable to active substances that have effects on the endocrine system.

However, the current EU legislation does not provide for a comprehensive, integrated assessment of cumulative effects taking into account different routes of exposure and different product types.

2 SUMMARY OF THE REPORT “STATE OF THE ART ASSESSMENT OF ENDOCRINE DISRUPTERS, 2ND INTERIM REPORT, PROJECT CONTRACT NUMBER 070307/2009/550687/SER/D3”

In 2002, the World Health Organization (WHO) published a first report³² on the state of knowledge on endocrine disruptors. Very recently, the European Commission wanted to update this knowledge and commissioned a consultant to undertake a new state-of-the-art assessment with the aim of possibly revising the regulations to be consistent with the state of science in this field. The first part of this work was discussed in a voluminous report³³ that was published on 31 January 2011. The summary presented below reviews its main points and conclusions, which reflect its authors' opinions.

2.1 Definitions of endocrine-disrupting (ED) chemicals – regulatory implications

The definitions published by national and international governmental agencies differ as to the use of 'adverse' effect and 'intact organism'. For example, the United States Environmental Protection Agency (US-EPA) does not consider endocrine disruption to be an adverse effect *per se*, but rather to be a mode or mechanism of action potentially leading to carcinogenic or reprotoxic effects, which are considered in reaching regulatory decisions. However, one of the main weaknesses of these definitions is that they are not anchored to specific assay outcomes. No tests can determine the potentially manifold and complex effects that might arise from endocrine disruption if defined by its mode of action. Although new tests are under development, in most cases, adequate model systems or assays are not available.

The WHO/IPCS definition that was approved internationally³⁴ was adopted as a working definition in the European Community Strategy for Endocrine Disruptors: “*An endocrine disrupter 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*”. An effort was also made to describe what a potential endocrine disruptor (ED) should be: “*A potential endocrine disrupter is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations*”. Furthermore, the definition of ‘adverse effect’ was adopted in the meeting organised by the German Federal Institute for Risk Assessment (BfR) in Berlin in November 2009: “*A change in morphology, physiology, growth, reproduction, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences*”.

To summarise, definitions that do not contain the notion of adverse effect are not adequately discriminatory and encompass a very large number of substances; conversely, definitions that introduce the notion of adverse effect raise questions regarding the very definition of 'adverse effect' in the context of the endocrine system which may be too restrictive and exclude substances that actually have endocrine-disrupting properties.

2.2 Emerging issues

Modulation of signalling pathways via their receptors

Nuclear receptors regulate numerous genes that are involved in cellular proliferation and differentiation, development, homeostasis and metabolism. Research has focused on the binding of ED substances to nuclear receptors for steroid hormones (oestrogen and androgen), which disrupts the binding of natural hormones, and to the aryl hydrocarbon receptor (Ahr), which regulates key

³² WHO/IPCS. Global assessment of the state-of-the-science of endocrine disruptors. WHO, 2002.

³³ State of the art assessment of endocrine disruptors. 2nd Interim Report, Project Contract Number 070307/2009/550687/SER/D3, PART 1 Summary of the state of the science. Richard Evans, Andreas Kortenkamp, Olwenn Martin, Rebecca McKinlay, Frances Orton, Erika Rosivatz (http://ec.europa.eu/environment/endocrine/documents/summary_state_science.pdf)

³⁴ Japan, USA, Canada, OECD and EU

metabolism enzymes (including P450 cytochromes). However, it is difficult to establish a link between the effects observed on the receptors and their potential consequences on human health; moreover, there are significant species differences. All of this has consequences on the development and choice of appropriate tests. Although numerous tests have been developed, only one OECD *in vitro* test (no. 455) has been validated for the detection of substances with oestrogenic agonist activity. Moreover, computational modelling tools are available (SAR, QSAR, etc.).

Low doses

The issue of low doses challenges the central dogma of conventional regulatory toxicology, which is that health effects that do not occur at high levels of exposure to a chemical cannot be induced by much lower levels of exposure to the chemical. In fact many studies, and particularly those undertaken with EDs, contradict this concept and there is a wealth of evidence that low-dose effects of EDs often cannot be predicted from high dose testing. Thus, current research combining toxicology, developmental biology, endocrinology and biochemistry has shown that some curves describing dose-response relationships are not linear but are expressed as 'U-shaped' or 'inverted-U-shaped' curves, reflecting an apparent reversal or inversion of the effect at a low region of the dose continuum; questions have therefore arisen about the appropriateness of assessing risks associated with EDs while assuming a linear dose-response relationship.

Multiple exposures

It is difficult to examine the effects of complex mixtures of substances in an experimental setting. The key aim of mixture toxicology is to anticipate quantitatively the effects of combinations of chemicals from knowledge about the effects of their individual components; the concepts of additivity, synergy and antagonism all come into play. The implications for risk assessment are significant. Here it is helpful to make a distinction between the cumulative approach (dose addition, independent action) and the specific approach for dealing with mixtures of EDs (hazard index, point of departure index and TEF³⁵ approach for dioxin combinations).

Epigenetic effects

Epigenetic mechanisms (DNA methylation, histone methylation, etc.) are responsible for genomic imprinting and form the basis of differential gene expression. It has been established that some EDs have the ability to alter the epigenome, which can cause trans-generational effects via epigenetic mechanisms. These epigenetic changes can occur throughout the lifetime of an organism and are mitotically heritable. However, it is important to discriminate between a chemical's ability to induce epigenetic changes and its ability to cause ED-type effects via epigenetic mechanisms.

Prostaglandins

Prostaglandins (especially PGD2) play an important role in regulating the differentiation of Sertoli cells, which are involved in male sexual development (SRY-SOX9-PGD2 cascade). It is plausible that disruption of this pathway by EDs causes testicular dysgenesis syndrome (TDS).

2.3 Effects on human health – Reproduction

Male reproductive health

Studies examining male reproductive health and exposure to EDs generally consider three groups of adverse effects:

- 1) decreases in semen quality and sub-fertility,
- 2) disruption of male foetal development resulting in malformations of the urogenital tracts (cryptorchidism, hypospadias, reduced anogenital distance),
- 3) testicular germ cell tumours.

In 2001, Skakkebaek *et al.*³⁶ suggested that these disorders, which mostly likely share a common

³⁵ TEF: Toxic Equivalent Factor

³⁶ Skakkebaek NE, Rajpert-De Meyts E, Main KM. 2001. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Human Reproduction* 16:972-978.

aetiological origin, constitute a syndrome termed testicular dysgenesis syndrome (TDS); under this hypothesis, diminished androgen action in male foetal life has a negative impact on the proper functioning of Sertoli cells and Leydig cells, which results in functional or organic reproductive abnormalities of varying severity. These authors also proposed environmental exposure to certain chemicals as the aetiological factor. Several studies later backed up these hypotheses, including studies on substances that modulate prostaglandins during pregnancy such as non-steroidal anti-inflammatory drugs, and studies on the occurrence of adverse effects on male reproductive system development.

Some epidemiological studies have shown relatively weak associations with certain individual chemicals. Furthermore, cumulative effects of simultaneous exposure have been shown, although methods for exploring this more thoroughly are currently lacking.

With the exception of testicular cancer, all of the components of TDS can be recapitulated in experimental animal models (OECD tests 414, 416), which are used to identify chemicals capable of inducing reproductive anomalies in rats. OECD test 443, an extended one-generation reproductive toxicity study in rats, examines relevant critical effects for the detection of chemicals that might interfere with male sexual development. The Hershberger *in vivo* test (no. 441) detects androgen receptor antagonist activity.

In vitro assays are available for the screening of substances that potentially have *in vivo* effects: androgen receptor antagonists, inhibition of steroidogenesis. An *in vitro* assay for the screening of prostaglandin D2 inhibitors is available, but has not been used widely. However, very little is known about correlations between *in vitro* activity and disruption of male sexual development, and *in vitro-in vivo* extrapolation is not currently possible.

Female reproductive health

There is no global mechanism that can explain the different disorders that affect female reproductive function: precocious puberty, fecundity and fertility disorders, polycystic ovarian syndrome, endometriosis and uterine fibroids.

Precocious puberty

The age of puberty is genetically determined but can also be influenced by environmental factors. Epidemiological studies undertaken in Europe and the USA have shown a downward trend for age at pubertal onset that is not due solely to the rise in obesity, which itself is suspected of being a consequence of the effects of EDs on foetal programming.

Epidemiological studies are limited due to the latency between exposure and puberty onset, the importance of the timing of exposure during the susceptible phases of foetal development and the establishment of diagnoses. Nonetheless, there is experimental evidence that exposure to xenobiotics in certain critical stages of foetal development can influence pubertal timing. OECD tests 416, 426 and 443 examine effects on pubertal onset.

Female fecundity

Fecundity encompasses a set of parameters related to a woman's ability to conceive: hormonal profile, menstruation, miscarriage, ovarian reserve, reproductive senescence and menopause. Recent epidemiological studies have highlighted the effects of certain environmental contaminants on several parameters of female fecundity. Short- and medium-term OECD tests such as nos. 407, 416 and 443 can be used to assess fecundity.

Polycystic ovarian syndrome

POS is a heterogeneous endocrine disorder considered the most common endocrine abnormality in women of childbearing age. Its complications include menstrual dysfunction, infertility, hirsutism, acne, obesity and metabolic syndrome. Although genetic factors play a role in this syndrome, the heterogeneity of phenotypic features even within the same family suggests the importance of the 'gestational environment' and lifestyle. While OECD tests 415 and 416 are theoretically capable of detecting substances acting via an androgenic mechanism, their sensitivity is too low to detect a potential increased risk in women who are genetically susceptible to developing this syndrome.



Fertility and adverse pregnancy outcomes

Adverse pregnancy outcomes include spontaneous abortion, ectopic pregnancies, foetal death, stillbirth, pre-term delivery, low birth weight, sex ratio and certain congenital defects. The literature from epidemiological research into environmental factors is abundant, but the primary difficulty is the inability to determine whether effects are caused by maternal or paternal exposure. OECD tests 400, 416 and 443 are useful in testing for these critical effects, with the exception of implantation loss, which is different in rodents.

Endometriosis

Endometriosis is a very common disorder characterised by ectopic endometrium with retrograde menstruation causing abdominal pain. It is believed to be due to the combined effects of genetic susceptibility, altered immune and hormonal response (excess oestrogen, progesterone) and environmental factors. There are at present no adequate experimental models that would allow the investigation of the effects of substances on the onset of endometriosis.

Uterine fibroids

Uterine fibroids are benign monoclonal tumours of the smooth muscle cells that can cause menorrhagia, abdominal pain, infertility and pregnancy complications. They are a leading cause of hysterectomy. The potential role of EDs in the onset of these hormone-dependent tumours is strongly suspected.

2.4 Effects on human health - hormone-dependent cancers

The role of EDs in the onset of hormone-dependent cancers such as breast, prostate, testicular and thyroid cancer is strongly suspected.

The current experimental approach used to identify substances that induce breast cancer is inadequate: the routinely used two-year rodent carcinogenicity assay lacks specificity to detect carcinogens with a hormonal mode of action, while the assays that are responsive to mammary carcinogens with an endocrine mode of action have not been investigated for their sensitivity. None of the *in vivo* tests currently proposed by the OECD for the testing of endocrine disruptors are able to identify mammary carcinogens.

Although the precise mechanisms by which certain substances are capable of inducing prostate cancer remain to be resolved, the key role of agents with androgenic and oestrogenic activity is strongly suspected in the aetiology of this cancer. More than ten animal models for prostate carcinogenesis have been described but none are able to recapitulate all of the features of this disease in men: androgen-dependence, developing androgen-independence at more advanced stages, slow growth with long latency periods and ability to metastasise to lymph nodes, bones and other organs. Moreover, in many rodent strains, including the F344 rat used for carcinogen testing, prostate tumours are not inducible by administration of androgens.

More than 90% of all tumours that afflict the testes are testicular germ cell tumours, of which 50% occur as seminomas. The risk factors that have been identified include hormonal imbalance and a lack of androgen action in foetal life; thus, exposure during critical windows of foetal life to substances with oestrogenic or anti-androgenic activity can increase the risk of this type of testicular tumour appearing. These tumours are rarely observed in rodents and the current animal models are not suitable.

Thyroid homeostasis can be altered by EDs through numerous mechanisms of action (direct effects, indirect effects on synthesis, secretion and the metabolism of thyroid hormones), which can lead to the onset of thyroid cancers. Animal models are not suitable because of rodent/human species differences; however, it appears that oestrogens play an important role, as more females suffer from the disease, and *in vitro* studies confirm that treatment with oestradiol increases the proliferation of thyroid cancer cell lines.

2.5 Effects on human health - metabolism and development

Developmental neurotoxicity

Disruption of thyroid function is the primary endocrine disruption mechanism responsible for neurotoxic effects due to the crucial role of thyroid hormones in the development of the central nervous system during foetal life and after birth in humans. The androgenic and oestrogenic signalling pathways are also involved in neurodevelopment since steroid receptors are expressed in the brain and could be targets of EDs. There are four OECD *in vivo* assays used for the exploration of neurotoxic effects, but only no. 426 is specific to developmental neurotoxicity.

Metabolic syndrome, obesity and diabetes

Metabolic syndrome includes insulin resistance, hyperinsulinaemia, hypertension and dyslipidaemia and is characterised by the presence of three out of five determinants: abdominal adiposity, hypertension, low high-density lipoprotein (HDL) cholesterol, elevated triglycerides and abnormal fasting glucose. The endocrine system is involved in the control of metabolism, which gives strong biological plausibility to the notion that EDs may be responsible for metabolic disruption through endocrine disruption. There are no or very few animal models but many *in vitro* models have been developed to test for the effects of substances on the nuclear receptors (oestrogens, androgens, glucocorticoids, peroxisome proliferator-activated receptor gamma [PPAR γ]), which are known to play an important role in the metabolic regulation of living organisms.

2.6 Ecotoxicology

Invertebrates

Knowledge of invertebrate endocrine systems is limited. However, studies in molluscs, arthropods and crustaceans show that the reproductive hormones of these organisms are similar in structure to those of vertebrates. Although their functions differ, the effects observed after their stimulation or inhibition can be extrapolated to vertebrates. The tests undertaken in these organisms can therefore be used to screen for relevant effects in vertebrates.

Effects such as the induction of imposex (development of male genital organs in females) and deformations of the shells of marine gastropods and bivalves have been observed.

In the context of pesticides, insect growth regulators, which have intentional endocrine effects, may have effects on the growth and development of non-target organisms, including vertebrates.

Fish

Sex differentiation in fish occurs during different stages according to the species and depends on environmental conditions such as temperature and nutrient levels. The following parameters are used to assess reprotoxic effects in fish:

- the gonadosomatic index associated with increased vitellogenin (VTG) synthesis and oestrogenicity
- gonadal histopathology, although the alterations occurring during spermatogenesis can be natural
- vitellogenin induction: a biomarker for exposure to oestrogens but with no evidence of association with reproductive impairment/histopathological effects. Moreover, VTG induction is dependent upon the season and temperature, etc. and hence is highly variable depending on sampling characteristics. Field extrapolation is therefore complex.
- egg production/fertilisation: a measurement that should be accompanied by histopathology in order to distinguish ED from general toxicity effects.

The growth and development of fish are controlled by growth hormones and thyroid hormones. The latter can affect reproductive capacities but the mechanism at play is not well understood. Hormonal induction can lead to:

- effects on the dominance of males, modifying their reproductive behaviour and decreasing the number of nests built,

- stress behaviour, resulting in loss of reproductive capability, weight loss and immune suppression. However, these effects have only been observed in adults and little is known about the actual effects on reproduction. It is therefore difficult to use these criteria for decision-making purposes.

There are several standardised screening and reproductive tests in fish (see Annex 1).

Amphibians

Gonadal differentiation during the larval stage can occur through three pathways:

- undifferentiated: all develop as females first then genetic males develop testicular tissue after metamorphosis,
- semi-differentiated: same as above but testicular tissue is developed during metamorphosis,
- differentiated: all develop as their genetic sex directly. The growth and development studies available have been undertaken in species with this type of development.

Differentiation can vary between populations in the same species that are separated geographically and it is therefore difficult to determine cause/effect relationships.

The thyroid gland is involved in metamorphosis and changes in its functioning can impact the completion of metamorphosis. Furthermore, oestrogens can inhibit metamorphosis through mechanisms that are not well understood.

The OECD tests listed in Annex 1 are screening tests that can highlight thyroid-disrupting effects.

Reptiles

The sex determination of reptiles depends on the genotype and temperature.

A decrease in the size of male reproductive organs in alligators and feminisation in turtles have been observed but could not be linked to endocrine disruptors. This was because no measurements of contaminants in the environment were available.

In-depth knowledge on the biology of reptiles and natural dimorphism would be necessary in order to understand the potential effects of certain substances.

Birds

During the sex differentiation of birds, the male phenotype is dominant and females rely on the synthesis of oestrogen by the ovaries during embryogenesis. Exposure to oestrogen can therefore stimulate the development of the female phenotype. Of the standardised tests in one and two generations of birds, the most suitable for assessing effects related to endocrine disruption is the two-generation test (see Annex 1).

The effects of DDT and other organochlorine pesticides on bird reproduction due to eggshell thinning have been demonstrated.

The thyroid gland plays a role in the growth and development of birds. Impairment of this gland and malformations have been observed in predatory birds.

Behavioural effects have also been observed, with decreased reproduction when single males mate with several females, decreased defending of nests and females spending less time incubating.

Mammals

The literature mentioned in the report focuses only on marine mammals belonging to declining populations, which are exposed for a long period during their juvenile phase due to their long lifespan, and whose feeding mode (building up stores of fat before the winter) promotes the accumulation of bio-accumulative pollutants.

Altered thyroid and growth hormone production has been correlated with concentrations of persistent organic pollutants (POPs). Likewise, levels of POPs in the blood and tissues have been

correlated with reproductive failure and adverse effects on the female reproductive system in pinnipeds and cetaceans.

There are no specific OECD tests for marine mammals. Nonetheless, tests in rodents can be useful. *In vitro* tests may be developed, as skin biopsies taken from various species of dolphins have been kept alive in culture.

3 PROPOSALS FROM MEMBER STATES, NON-GOVERNMENTAL ORGANISATIONS AND INDUSTRY ON THE DEFINITION OF ENDOCRINE DISRUPTION

3.1 *Proposals of certain Member States*

3.1.1 Summary of the joint Germany/United Kingdom³⁷ proposal on the regulatory definition of an endocrine disruptor in relation to potential effects on human health

A substance shall be considered as an endocrine disruptor and cannot be approved under Regulation (EC) no. 1107/2009 on the marketing of plant protection products (unless exposure is negligible) when it meets the following definition (proposed by the WHO in 2002) and the related criteria.

It should be an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse effects in an intact organism, or its progeny, or (sub)populations and satisfies the following criteria:

- Adverse effects have been seen in one or more toxicity studies of acceptable quality, in which the substance was administered by a route relevant for human exposure.
- There is a plausible mode-of-action/mechanistic link between the toxic effects of concern and endocrine disruption.
- The effects seen in experimental animals are judged as relevant to human health.
- Serious adverse effects related to endocrine disruption have been produced at a dose at or below the relevant guidance value for application of Specific Target Organ Toxicity – Repeated Exposure Category 1 (STOT-RE 1) classification under Regulation (EC) no. 1272/2008 on classification, labelling and packaging of substances and mixtures.

3.1.2 Summary of the Denmark³⁸ proposal on the establishment of criteria for endocrine disruptors and options for regulation

Substances are divided into two groups:

- **Category 1: confirmed endocrine disruptors**

This category corresponds to the WHO's definition of endocrine disruptor (see above). The substances placed in this category cannot be approved under Regulation (EC) no. 1107/2009 (unless exposure is negligible).

Substances are placed in category 1 when they are known to have caused ED-mediated adverse effects in humans or animal species living in the environment or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to cause adverse ED effects in humans or animals living in the environment.

The animal studies shall provide clear evidence of ED effects in the absence of other toxic effects, or if occurring together with other toxic effects, the ED effects should be considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans or the environment, category 2a may be more appropriate.

Substances can be allocated to this category based on:

- adverse *in vivo* effects where an ED mode of action is highly plausible;
- ED mode of action *in vivo* that is clearly linked to adverse effects *in vivo* (e.g. by read-across).

³⁷ Joint DE-UK position paper: Regulatory definition of an endocrine disrupter in relation to potential threat to human health, BfR, May 2011

³⁸ *Establishment of criteria for endocrine disruptors and options for regulation*, Danish Ministry of the Environment, Environmental Protection Agency, May 2011

- **Category 2: potential endocrine disruptors**

The definition of potential endocrine disruptors, also proposed by the WHO, is as follows: a potential endocrine disruptor is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations. This category is divided into two sub-categories: category 2a for suspected EDs and category 2b for substances with indications of ED properties. Further data must be provided in order for these substances placed in category 2a or 2b to be authorised under Regulation (EC) no. 1107/2009.

- **Category 2a: suspected endocrine disruptors**

Substances are placed in category 2a when there is some evidence for ED effects from humans or experimental animals, and where the evidence is not sufficiently convincing to place the substance in category 1.

These endocrine-disrupting effects should be observed in the absence of other toxic effects, or if occurring together with other toxic effects, the ED effect should be considered not to be a secondary non-specific consequence of other toxic effects.

Substances can be allocated to this category based on:

- adverse effects *in vivo* where an ED mode of action is suspected;
- ED mode of action *in vivo* that is suspected to be linked to adverse effects *in vivo* (e.g. by read-across);
- ED mode of action *in vitro* combined with toxicokinetic *in vivo* data (and relevant non-test information such as read-across, chemical categorisation and (Q)SAR predictions).

- **Category 2b: substances with indication of endocrine-disrupting properties**

Substances are placed in category 2b when there is some *in vitro/in silico* evidence indicating a potential for endocrine disruption in intact organisms.

The evidence could also be observed effects *in vivo* where there is general but not specific evidence relating those to ED (general but not specific effects).

3.2 Summary of proposals of Non-Governmental Organisations

3.2.1 Summary of the proposal of the Pesticide Action Network Europe³⁹ on pesticides with endocrine-disrupting properties

A substance should be considered as having endocrine-disrupting properties, and cannot be authorised as an active substance by virtue of the provisions of Regulation (EC) no. 1107/2009, when effects on the endocrine system are observed, including effects secondary to other toxic effects. A known mechanism of action is not necessary.

The approach should be based on hazard and not risk assessment.

An in-depth review of the scientific literature should be undertaken for a hazard assessment. Data from independent organisations are preferred.

Independent scientists working on endocrine disruption should develop a modern study protocol. In order to identify substances having endocrine-disrupting properties, it is necessary to study all hormonal systems, perform low-dose testing, consider the notion of exposure window and thus administer the substance to animals during their development.

Regarding the interpretation of study results, the effects observed in animals should by default be considered relevant for humans. The notion of threshold should not be used for endocrine-disrupting properties. If there is doubt about the adverse effects of chemicals with endocrine-disrupting properties, the precautionary principle must be used and the chemical withdrawn from the market until further studies are evaluated.

³⁹ PAN Europe position paper on criteria for endocrine disrupting pesticides, May 2011

3.2.2 Summary of the proposals of the Chemical Health and Environment Monitoring Trust and WWF European Policy Office⁴⁰ on the regulation of active substances with endocrine-disrupting properties under REACH and the pesticide regulations

In order to identify substances with endocrine-disrupting properties, the proposed criteria are similar to those developed by PAN Europe. A classification of these substances is also proposed.

Substances are divided into four categories: The substances placed in category 1 (1A, 1B and 1C) are considered as having endocrine-disrupting properties. These substances should not be approved under Regulation (EC) no. 1107/2009 and should be replaced by substances that do not have endocrine-disrupting properties.

- **Category 1**

- **Category 1A**

Substances are placed in category 1A when there is enough evidence to be sure that the adverse effect is a direct consequence of disruption of the endocrine system. The causal mechanism has therefore been established with certainty.

- **Category 1B**

Category 1B could be applied to substances as defined in category 1A, but where the causal mechanism is not known with certainty, although the adverse effect is strongly suspected to be mediated via disruption of the endocrine system.

- **Category 1C**

Category 1C could be applied to substances as defined in category 1B, but where there is less evidence for endocrine-mediated effects, and/or where endocrine disruption is strongly suspected or known but where there is debate over whether the effects reported should be considered adverse. It would also include substances considered as having endocrine-disrupting properties *in vivo* (e.g. effects on hormone levels, hormone-sensitive tissues, endocrine glands, auxiliary systems).

- **Category 2**

Substances are placed in category 2 where there are suspected endocrine-disrupting effects on the basis of *in vitro* tests (e.g. receptor binding assays) or non-validated QSAR models, unless there are sufficient data to negate the concerns.

3.3 Industry proposal (European Centre for Ecotoxicology and Toxicology of Chemicals⁴¹): Guidance document on the identification of endocrine-disrupting effects

It is considered that there is evidence of endocrine-disrupting properties when the adverse effects observed in regulatory toxicology studies may be explained by screening/mechanistic studies, or vice-versa, when the indications of endocrine-disrupting activity observed in screening/mechanistic studies may be confirmed by adverse effects found in regulatory toxicology studies. Various factors can be used to discriminate between endocrine disruptors according to their level of concern: relevance of the endocrine-disrupting mechanism of action to humans, specificity of endocrine effects in relation to other potential toxic effects, potency of the substance to induce endocrine toxicity and exposure level.

A decision tree was developed in order to determine, using the various study types available for a substance, whether it has endocrine-disrupting properties, according to the definition of an endocrine disruptor issued at Weybridge [UK] in 1996: an endocrine disruptor is an exogenous

⁴⁰ CHEM Trust's Contribution to the Ongoing Debate on Criteria for EDCs, September 2011 and CHEM Trust and WWF-EPO proposals for the regulation of chemicals with endocrine disrupting properties under REACH (EC 1907/2006) and under the Plant Protection Products Regulation (EC no 1107/2009), December 2010

⁴¹ *Guidance on Identifying Endocrine Disrupting Effects*, ECETOC Technical Report n°106, June 2009 and Bars R. et al., *Science based guidance for the assessment of endocrine disrupting properties of chemicals*, Regulatory Toxicology and Pharmacology 59 (2011) 37-46

substance that causes adverse effects in an intact organism, or its progeny, secondary to changes in endocrine function.

- **Scenarios A, B, D and E:** no or insufficient evidence of endocrine-disrupting effects
 - **Scenario A:** no adverse effects on endocrine activity in general *in vivo* toxicology studies
 - **Scenario B:** no adverse effects on endocrine activity in general *in vivo* toxicology studies and positive results in specific studies (mechanistic, *in vitro* or *in vivo*)
 - **Scenario D:** adverse effects on endocrine activity in general *in vivo* toxicology studies and no evidence of endocrine-disrupting activity in specific studies (mechanistic, *in vitro* or *in vivo*)
 - **Scenario E:** in the absence of other data, negative results in specific studies (mechanistic, *in vitro* or *in vivo*)
- **Scenario C:** sufficient evidence of endocrine-disrupting effects in laboratory animals
Adverse effect(s) on endocrine activity in general *in vivo* toxicology studies and evidence of endocrine-disrupting activity in mechanistic studies (*in vitro*, *in vivo*)

The next step is to consider this effect's specificity, relevance to humans and potency:

- If the adverse effect is not specific or is specific but not relevant to humans, the risk assessment will be based on effects that are not endocrine disrupting. A specific adverse effect is defined as an adverse effect on the endocrine system occurring at dose levels lower than any other forms of adverse effects (e.g. neuro-, hepato-, cardio-toxicity).
- If the adverse effect is specific and relevant to humans, and exposure is not negligible, the risk assessment will be based on the endocrine-disrupting effect(s), applying variable uncertainty factors based on the potency of the effect. The potency of an effect depends on several factors: dose at which the effect occurs, duration of exposure required to induce the effect, type and severity of endocrine effects, number of species affected.

Second Part: Possible consequences of regulatory changes in the assessment of authorisations for plant protection products

1 LIST OF CURRENTLY APPROVED ACTIVE SUBSTANCES THAT COULD SATISFY THE NON-APPROVAL CRITERIA SET OUT IN POINTS 3.6.2, 3.6.3 AND 3.6.4 OF REGULATION (EC) NO. 1107/2009 WHEN THEIR APPROVAL IS RENEWED

On the basis of the available assessment reports on active substances and the regulatory data [particularly harmonised EU classifications and the French national classification (Agritox, October 2011⁴²)], it is possible to establish a provisional list of active substances approved under Regulation (EC) no. 1107/2009 that, when their approval is renewed, will no longer satisfy the approval criteria set out in points 3.6.2, 3.6.3 and 3.6.4 of Annex II to this Regulation (presented in Annex 2). It should be noted that this list does not take into account all of the approval criteria set out in Annex II of the Regulation.

The list given below should be regarded as provisional and takes into account only active substances for which a product is authorised in France. It is based on the approval criteria proposed in the Regulation, which take into account the intrinsic properties of substances. Some criteria are provisional, such as those related to endocrine disruption.

Table 1: Active substances that satisfy the criteria in Annex II, points 3.6.2, 3.6.3 and 3.6.4

Substance	Old classification	New classification
Flumioxazin	Repr. Cat. 2 R61 *	Repr1B H360D *
Flusilazole	Carc. Cat. 3 R40 Repr. Cat. 2 R61 *	Carc2 H351 Repr1B H360D *
Glufosinate	Repr. Cat. 2 R60 Repr. Cat. 3 R63 *	Repr1B H360Fd STOT Re2 H373 *
Linuron	Carc. Cat. 3 R40 Repr. Cat. 2 R61 Repr. Cat. 3 R62 *	Carc2 H351 Repr1B H360Df STOT Re2 H373 *

* Harmonised classification (Regulation (EC) no. 1272/2008)

2 LIST OF ACTIVE SUBSTANCES APPROVED OR IN THE COURSE OF BEING APPROVED, UNLIKELY TO SATISFY THE 'DEFAULT' CRITERIA IN REGULATION (EC) NO. 1107/2009 WITH REGARD TO ADVERSE ENDOCRINE-DISRUPTING EFFECTS IN HUMANS AND NON-TARGET ORGANISMS WHEN THEIR APPROVAL IS RENEWED

On the basis of the available assessment reports on active substances and the regulatory data [particularly harmonised EU classifications and the French national classification (Agritox, October 2011)], it is possible to establish a provisional list of active substances approved or under approval according to Regulation (EC) no. 1107/2009 that, when their approval is renewed, may not satisfy the 'default' approval criteria set out in point 3.6.5 of Annex II to this Regulation and point 3.8.2 (presented in Annex 2). It should be noted that this list does not take into account all of the approval criteria set out in Annex II of the Regulation.

The list given below should be regarded as provisional and takes into account only active substances for which a product is authorised in France. It is based on the approval criteria proposed in the Regulation, which take into account the intrinsic properties of substances. Some criteria are provisional, such as those related to endocrine disruption.

⁴² <http://www.dive.afssa.fr/agritox/index.php>

Table 2: Active substances that satisfy the 'default' criteria in Annex II point 3.6.5 (active substances...considered to have endocrine-disrupting properties that may cause adverse effects in humans...) and Annex II point 3.8.2 (active substances...considered to have endocrine-disrupting properties that may cause adverse effects on non-target organisms...)

Substance	Old classification	CLP classification	Target organ
Aminotriazole	Repr. Cat. 3 R63 *	Repr2 H361d *	Thyroid. Testicles.
Carbetamide	Repr. Cat. 3 R63 Carc. Cat 3 R40 [§]	Repr2 H361d Carc2 H351 [§]	Liver (histopathological changes). Haematological effect. Thyroid. Increased gestational period. Foetotoxicity.
Chlorotoluron	Repr. Cat. 3 R63 Carc. Cat. 3 R40 *	Repr2 H361d Carc2 H351 *	Liver (histopathological changes). Haematological effects. Adrenal glands. Resorption and foetotoxicity.
Cyproconazole	Repr. Cat. 3 R63 *	Repr2 H361d *	Liver (histopathological changes). Foetal abnormalities at maternotoxic doses and increased post-implantation loss.
Dimoxystrobin	Repr. Cat. 3 R63 Carc. Cat 3 R40 *	Repr2 H361d Carc2 H351 *	Intestines. Foetotoxicity
Epoxiconazole	Carc. Cat 3 R40 Repr. Cat. 3 R62 R63 *	Carc2 H351 Repr2 H361fd *	Liver, adrenal glands, ovaries and effects on hormonal regulation.
Fluazifop-P-butyl	Repr. Cat. 3 R63 *	Repr2 H361d *	Liver. Kidneys. Spleen. Eyes. Haematological effects. Weight variation: spleen, testicles, epididymis, pituitary gland, uterus. Delayed foetal development.
Flurochloridone	Repr. Cat. 3 R62 [§]	Repr2 H361f [§]	Liver, testicles, epididymis. Decreased sperm count.
Ioxynil	Repr. Cat. 3 R63 *	Repr2 H361d *	Liver (histopathological changes). Thyroid.
Mancozeb	Repr. Cat. 3 R63 *	Repr2 H361d *	Thyroid.
Maneb	Repr. Cat. 3 R63 *	Repr2 H361d *	Thyroid.
Metconazole	Repr. Cat. 3 R63 *	Repr2 H361d *	Liver (histopathological changes). Adrenal glands. Resorption and foetotoxicity
Myclobutanil	Repr. Cat. 3 R63 *	Repr2 H361d *	Testicles (atrophy). Liver (histopathological changes).
Oxadiazyl	Repr. Cat. 3 R63*	Repr1A H360Fd *	Liver (histopathological changes). Haematological effects. Thyroid.
Pinoxaden	Repr. Cat. 3 R63 [§]	Repr2 H361d [§]	Liver (histopathological changes). Kidneys. Uterus. Defects and foetotoxicity.
Prothioconazole	Repr. Cat. 3 R63 [§]	Repr2 H361d [§]	Prothioconazole: liver and kidney (nephropathy). Desthio-prothioconazole, a metabolite of prothioconazole in plants and animals, has effects on the endocrine system.
Quinoclamine	Repr. Cat. 3 R63 *	Repr2 H361d *	Liver (histopathological changes). Haematological effects. Adrenal glands. Kidneys. Defects.
Tebuconazole	Repr. Cat. 3 R63 *	Repr2 H361d *	Liver and effects on hormonal regulation.
Tembotrione	Repr. Cat. 3 R63 [§]	Repr2 H361d [§]	Liver (histopathological changes). Pancreas. Kidneys. Thyroid. Defects and foetotoxicity.

* Harmonised classification (Regulation (EC) no. 1272/2008).

§ Non-harmonised classification, consolidated presentation in the Agritox database. The analysis took into account the classifications of October 2011. These classifications are subject to change according to the results of national and European assessments and the publication of harmonised classifications.

This list should therefore be revised to take into account the conclusions adopted in Europe and a guidance document on endocrine-disrupting effects. That document will clarify the criteria used. The above list may expand.

Effects of endocrine disruptors on non-target organisms

The active substances in Table 2, considering their target organs and reported effects, may also satisfy the criteria in Annex II point 3.8.2. (active substances...considered to have endocrine-disrupting properties that may cause adverse effects on non-target organisms...).

However, no criteria have been defined to consider an active substance as having endocrine-disrupting properties that may cause adverse effects on non-target organisms in the wild fauna. Many questions remain to be answered before these criteria can be defined.

First, there is a lack of in-depth knowledge into the functioning of certain species, such as reptiles, whose biology is not well understood. For example, data on natural variations in sexual dimorphism are insufficient. Research will therefore be necessary to improve this knowledge and make it easier to interpret results and make decisions regarding the potential effects of endocrine disruptors on populations of wild animals.

Moreover, in some cases it is not possible to establish a relationship between the causes of disruption and effects observed *in situ*. It is therefore difficult or even impossible to extrapolate effects observed in the laboratory setting to the field. This is true for certain effects observed in the laboratory setting in fish (e.g. vitellogenin assay) and effects that impact animal behaviour that is difficult to study in the field, for example that of marine mammals.

Furthermore, when certain effects are observed in the laboratory setting they can lead to false positives if no precautions are taken in the interpretation of results. Indeed, some parameters can vary in the field due to natural alterations (e.g. spermatogenesis). It is therefore difficult to distinguish between these natural alterations and disruptions caused by chemical compounds. Thus, if an effect is observed, a compound may wrongly be considered as an endocrine disruptor. Supplementary studies may be required in order to avoid incorrect interpretations due to a lack of data.

3 IMPACT OF THE PROPOSALS MENTIONED IN PART 1 SECTION 3: CASE STUDY APPLIED TO 11 ACTIVE SUBSTANCES

A classification of 11 active substances was established using the criteria proposed by certain Member States, NGOs and industry (ECETOC), based on the available assessment reports, regulatory data (particularly harmonised classifications of substances) and the literature. These active substances were selected for their presumed endocrine-disrupting potential. However, considering the time that was required to process this Request, the list given in Table 3 shows only a sample of these active substances that are suspected endocrine disruptors and is therefore not an exhaustive list of plant protection products.

The criteria proposed in the various papers (Member States, NGOs, industry) leave considerable room for expert opinion. The results shown in Table 3 are based on toxicological data and mechanisms of action, which were used to classify the active substances into various categories.

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Table 3: Case study results

A substance cannot be authorised under Regulation (EC) no. 1107/2009 if it belongs to the following category:

- Category 1 according to the DK proposal
- Categories 1A, 1B, 1C according to the CHEM Trust/WWF proposal

Substance	Old classification	New classification	Results according to the various proposals on the definition of endocrine disruption				
			DE-UK proposal	DK proposal	NGO proposals		Industry proposal (ECETOC)
					CHEM Trust /WWF	PAN- Europe	
Myclobutanil CAS: 88671-89-0	Xn, Repr. Cat. 3 R63 R22 R36 *	Acute toxicity, Cat 4, H302 Eye irritation, Cat 2, H319 Reproductive toxicity, Cat 2, H361d *	Yes	Cat. 1	Cat. 1A	Yes	Scenario C (risk assessment based on endocrine endpoint with a suitable safety factor)
Flusilazole CAS: 85509-19-9	T, Carc. Cat. 3 R40 R22 R61 *	Acute toxicity, Cat 4, H302 Carcinogenicity, Cat 2, H351 Reproductive toxicity, Cat 1B, H360D * *	Yes	Cat. 1	Cat. 1A	Yes	Scenario C (risk assessment based on endocrine endpoint with a suitable safety factor)
Iprodione CAS: 36734-19-7	Xn, Carc. Cat. 3 R40 *	Carcinogenicity, Cat 2, H351 *	No	Cat. 1	Cat. 1A	Yes	Scenario C (risk assessment based on endocrine endpoint with a suitable safety factor)
Propyzamide CAS: 23950-58-5	Xn, Carc. Cat. 3 R40 *	Carcinogenicity, Cat 2, H351 *	No	Cat. 1	Cat. 1A	Yes	Scenario C (risk assessment based on endocrine endpoint with a suitable safety factor)
Linuron CAS: 330-55-2	T, Carc. Cat. 3 R40 Repr. Cat. 2 R61 Repr. Cat. 3 R62 R22 R48/22 *	Acute toxicity, Cat 4, H302 Carcinogenicity, Cat 2, H351 Reproductive toxicity, Cat 1B(Df), H360Df Specific target organ toxicity – Repeated exposure, Cat 2, H373 *	Yes	Cat. 1	Cat. 1A	Yes	Scenario C (risk assessment based on hormonal effects with a suitable safety factor)

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Substance	Old classification	New classification	Results according to the various proposals on the definition of endocrine disruption				
			DE-UK proposal	DK proposal	NGO proposals		Industry proposal (ECETOC)
					CHEM Trust /WWF	PAN-Europe	
Triflurosulfuron-methyl CAS: 126535-15-7	Xn, Carc. Cat. 3 R40 §	Carcinogenicity, Cat 2, H351 §	No	Cat. 1	Cat. 1A	Yes	Scenario C (risk assessment based on endocrine endpoint with a suitable safety factor)
Chlorsulfuron CAS: 64902-72-3	Not classified *	Not classified *	No	Cat. 1	Cat. 1A	Yes	Scenario C (risk assessment based on endocrine endpoint with a suitable safety factor)
Propargite [§] CAS: 2312-35-8	T, Carc. Cat. 3 R40 R23 R38 R41 *	Acute toxicity, Cat 3, H331 Carcinogenicity, Cat 2, H351 Serious eye damage, Cat 1, H318 Irritating to the skin, Cat 2, H315 *	No	Cat. 2B	Cat. 1B	Yes	Scenario D
Glyphosate CAS: 1071-83-6	Xi, R41 *	Serious eye damage, Cat 1, H318 *	No	No	No	Yes	Scenario E
Imazalil CAS: 3554-44-0	Xn, R20/22 R41 *	Acute toxicity, Cat 4, H302 Acute toxicity, Cat 4, H332 Serious eye damage, Cat 1, H318 *	No	Cat. 2B	Cat. 2	Yes	Scenario B
Carbetamide CAS: 16118-49-3	Xn, Carc. Cat. 3 R40 Repr. Cat. 3 R63 R22 §	Acute toxicity, Cat 4, H302 Carcinogenicity, Cat 2, H351 Reproductive toxicity, Cat 2, H361d §	No	Cat. 2B	Cat. 1B	Yes	Scenario D

* Harmonised classification (Regulation (EC) no. 1272/2008).

§ Non-harmonised classification, consolidated presentation in the Agritox database. The study took into account the classifications of October 2011. These classifications are subject to change according to the results of national and European assessments and the publication of harmonised classifications.

§ Unapproved substance

Conclusions

The information presented in this report illustrates the state of current European thinking on how endocrine disruptors should be addressed in the regulations.

Focusing more specifically on plant protection products, the hazards related to the active substances in these products are assessed based on regulatory studies undertaken according to internationally recognised protocols and the data available in the literature. Moreover, protocols have been developed by the OECD to more specifically assess the endocrine-disrupting effects of substances. In the case of active substances contained in plant protection products, further investigations could, depending on the type of effect observed in animals, prove necessary to identify the mechanisms of action that cause these effects and thereby confirm or refute the existence of a threshold.

Regarding the possible consequence of these changes in the system for the assessment of plant protection product authorisations, two types of analyses were undertaken.

First, an analysis of the consequences was undertaken based on the approval criteria in Regulation (EC) no. 1107/2009 (Annex II; points 3.6.2, 3.6.3, 3.6.4 and 3.6.5). The criteria for the identification of substances having endocrine-disrupting effects (point 3.6.5) were set 'by default' and still need to be specified. This analysis showed that the default criteria are not entirely suitable for identifying substances having endocrine-disrupting effects. Moreover, there is a lack of knowledge of potential effects on certain species in the environment, particularly on how to take into account natural variations. Therefore, research needs to be developed to improve this knowledge and make it easier to interpret results and make decisions regarding the potential effects of endocrine disruptors on wild populations.

A case analysis was also undertaken in the light of the proposals of certain Member States, NGOs and industry (ECETOC) on the definition of endocrine disruption. This case study confirmed the significant role of expert opinion in the analytical process. This conclusion underlines the importance of defining unequivocal decision-making criteria. However, this study should still be supplemented in order to make it more exhaustive and better characterise impacts. It could be combined with an analysis of the potential benefits (primarily agricultural) of these active substances compared to their consequences on human health and the environment. Furthermore, while hazards related to active substances can be identified through the available studies, knowledge of exposure remains fundamental to determine risks.

Marc Mortureux
Director-General

Annex 1

List of OECD Test Guidelines

Health effects

- OECD 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents
- OECD 414: Prenatal Developmental Toxicity Study
- OECD 415: (Extended) One-Generation Reproduction Toxicity Study
- OECD 416: Two-Generation Reproduction Toxicity Study
- OECD 426: Developmental Neurotoxicity Study
- OECD 440: Uterotrophic Bioassay in Rodents
- OECD 441: Hershberger Bioassay in Rats
- OECD 443: Extended One-Generation Reproductive Toxicity Study in Rats
- OCDE 453: Combined Chronic Toxicity/Carcinogenicity Studies
- OECD 455: Stably Transfected Human Estrogen Receptor- α Transcriptional Activation Assay for the Detection of Estrogenic Agonist-Activity of Chemicals
- OECD 456: H295R Steroidogenesis Assay

Screening tests in the wild fauna

- OECD 229: Fish Short Term Reproduction Assay
- OECD 230: 21-Day Fish Assay
- Variant of OECD 230: Androgenised Female Stickleback Screen
- OECD 231: Amphibian Metamorphosis Assay
- OECD 206: Avian Reproduction Test
- OECD 234: Fish Sexual Development Test

Draft guidelines for the wild fauna

- Fish (Medaka) Multi-Generation Test
- Larval Amphibian Growth and Development Assay
- Avian Two Generation Test

Annex 2

Extract from Annex II of Regulation (EC) no. 1107/2009

Procedure and criteria for the approval of active substances, safeners and synergists pursuant to Chapter II

Point 3.6.2. An active substance, safener or synergist shall only be approved if, on the basis of assessment of higher tier genotoxicity testing carried out in accordance with the data requirements for the active substances, safeners or synergists and other available data and information, including a review of the scientific literature, reviewed by the Authority, it is not or has not to be classified, in accordance with the provisions of Regulation (EC) No. 1272/2008, as mutagen category 1A or 1B.

Point 3.6.3. An active substance, safener or synergist shall only be approved, if, on the basis of assessment of carcinogenicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, it is not or has not to be classified, in accordance with the provisions of Regulation (EC) No. 1272/2008, as carcinogen category 1A or 1B, unless the exposure of humans to that active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No. 396/2005.

Point 3.6.4. An active substance, safener or synergist shall only be approved, if, on the basis of assessment of reproductive toxicity testing carried out in accordance with the data requirements for the active substances, safener or synergist and other available data and information, including a review of the scientific literature, reviewed by the Authority, it is not or has not to be classified, in accordance with the provisions of Regulation (EC) No. 1272/2008, as toxic for reproduction category 1A or 1B, unless the exposure of humans to that active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with Article 18(1)(b) of Regulation (EC) No. 396/2005.

Point 3.6.5 An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines or other available data and information, including a review of the scientific literature, reviewed by the Authority, it is not considered to have endocrine-disrupting properties that may cause adverse effect in humans, unless the exposure of humans to that active substance, safener or synergist in a plant protection product, under realistic proposed conditions of use, is negligible, that is, the product is used in closed systems or in other conditions excluding contact with humans and where residues of the active substance, safener or synergist concerned on food and feed do not exceed the default value set in accordance with point (b) of Article 18(1) of Regulation (EC) No. 396/2005.

By 14 December 2013, the Commission shall present to the Standing Committee on the Food Chain and Animal Health a draft of the measures concerning specific scientific criteria for the determination of endocrine-disrupting properties to be adopted in accordance with the regulatory procedure with scrutiny referred to in Article 79(4). Pending the adoption of these criteria, substances that are or have to be classified, in accordance with the provisions of Regulation (EC) No. 1272/2008, as carcinogenic category 2 and toxic for reproduction category 2, shall be considered to have endocrine-disrupting properties. In addition, substances, such as those that are or have to be classified, in accordance with the provisions of Regulation (EC) No. 1272/2008, as toxic for reproduction category 2 and which have toxic effects on the endocrine organs, may be considered to have such endocrine-disrupting properties.

Point 3.8.2. An active substance, safener or synergist shall only be approved if, on the basis of the assessment of Community or internationally agreed test guidelines, it is not considered to have endocrine-disrupting properties that may cause adverse effects on non-target organisms unless the exposure of non-target organisms to that active substance in a plant protection product under realistic proposed conditions of use is negligible.