Gezondheidsraad
Health Council of the Netherlands

To the State Secretary for Infrastructure and the Environment

Subject	: Presentation of advisory letter *Tests for Chemical Substances*
Your reference	: IENM/BSK-2012/178475
Our reference	: I-1349/12/SD/fs/873-G1 Publication no. 2012/34E
Enclosure(s)	: 2
Date	: December 14, 2012

Dear State Secretary,

On 26 September 2012 your predecessor asked the Health Council of the Netherlands (see Annex A) whether it would be desirable, considered within the context of the European REACH (Registration, Evaluation, Authorisation and Restriction of Chemical substances) regulation, to replace the current *Two-Generation Reproduction Toxicity Study (TGRTS)* by the *Extended One-Generation Reproduction Toxicity Study (EOGRTS).* The Health Council's Committee on Prenatal Substance Exposure (see Annex B) has considered this question at my request. Its findings are contained in this memorandum.

Background

Various tests involving experimental animals are used to assess the safety of chemical substances. Since the 1980s, the *Two-Generation Reproduction Toxicity Study* (TGRTS, OECD test guideline 416)[1] has been the one most widely internationally accepted within the OECD for detecting the effects of substance exposure on reproduction. This test covers all phases of the reproductive cycle. Exposure to the substance under test starts with adult male and female experimental animals, and is continued successively during mating, gestation, birth and up to weaning. Exposure to the test substance is continued further to first generation offspring during their growth into adulthood, mating and production of a second generation (until the weaning).

A need was felt for a new test for a number of reasons, including the large number of experimental animals required for the TGRTS. The initial proposal for the *Extended One-Generation Reproduction Toxicity Study* was made in 2006.[2] The EOGRTS studies one generation less than the TGRTS, which leads to a substantial reduction in the number of experimental animals used

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(from about 2,600 to 1,400 experimental animals per test). Another reason why a new test was required was the limited predictive power of the TGRTS for (developmental) neuro- and immune-toxic effects. The EOGRTS comprises a large number of additional parameters in order to increase this predictive power. The parameters for the neurotoxic effects are derived from an existing test protocol (OECD test guideline 426).[3] The parameters for the immunotoxic effects are widely used in studies on adult animals, but have not yet been used in reproduction toxicity studies. Furthermore, more parameters giving information about possible endocrine disturbances due to substance exposure are measured in de EOGRTS (the TGRTS already had a number of parameters for this purpose). The EOGRTS has been further developed since 2006 with the aid of a number of workshops, expert meetings and publications,[4,5,6] resulting in the publication of an OECD guideline for the EOGRTS (OECD test guideline 443) in 2011.[7]

Protection level offered by the new test

An analysis of 498 available TGRTSs was carried out in a large international study,[5] in which the sensitivities to the various test substances determined by the full TGRTS were compared with those found when the results for the second generation offspring were neglected. No difference was found between the (values of the) "no observed adverse effect level" (NOAEL) determined by the two methods.

A second international analysis also found that consideration of the effects observed in the second generation offspring made no difference to the results.[6] This study concerned the classification and labelling of the reproductive toxicity of fifty substances (on the basis of the current European guideline) for which TGRTS data were available.

These analyses show that the TGRTS gives no added value compared with the EOGRTS in practice as regards both risk evaluation and classification and labelling.

As mentioned above, the EOGRTS contains additional factors not tested for in the TGRTS. This set of neurotoxicity and immunotoxicity parameters, which are so far not regularly measured within the REACH assessment framework, would permit improved detection of adverse effects of pre- and postnatal exposure. Studies carried out in the meantime (for example those of Raffaele et al. and Tonk et al.)[8,9] have confirmed the relative sensitivity of these parameters. This could lead to a higher level of protection for humans in the case of substances for which these parameters are most sensitive. The protection level is raised further by the fact that the additional parameters are

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determined in animals born during the test. As a result, the statistical power, and hence also the protection level, of the test are increased without the need for more experimental animals.

Recommendation

In brief, the Committee concludes that the EOGRTS offers a level of health protection that is at least as high as that of the TGRTS. In addition, the EOGRTS gives clear added value because it also tests the effects of exposure to hazardous substances on the development of the nervous system, the immune system and the endocrine system. The Committee therefore recommends the introduction of the EOGRTS.

Having heard the advice of the Standing Committee on Health and the Environment and the Subcommittee on Classification of Reprotoxic Substances, I hereby confirm the conclusions and recommendations of the Committee on Prenatal Substance Exposure.

Yours sincerely,
(signed)
Professor H. Obertop
Vice President

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Literature

1 OECD Test Guideline 416. OECD Guideline for Testing of Chemicals. Two-generation Reproduction Toxicity Study. Organisation for Economic Co-operation and Development, Paris, France; 2001.

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4 Cooper RL. Current developments in reproductive toxicity testing of pesticides. Reproductive Toxicology 28 (2009) 180–187.

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7 OECD, 2012. Test No. 443: Extended One-Generation Reproductive Toxicity Study OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects.

8 Raffaele,K.C.; Rowland,J.; May,B.; Makris,S.L.; Schumacher,K.; Scarano,L.J. The use of developmental neurotoxicity data in pesticide risk assessments. Neurotoxicol. Teratol 2010; 32:563-572.

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Annex **A**

Request for advice

Date of request: 26 September 2012,
Our ref.: IENM/BSK-2012/178475

Dear President,

The purpose of this letter is to ask your advice on the use of a new test for assessment of the safety of chemical substances.

The safety of chemical substances for health and the environment is a matter of great importance to society. A European regulation (REACH) laying down requirements for the safe use of chemical substances was therefore approved in 2006. The producers of such substances or those who put them on the market have an obligation to ensure that these substances can be used safely. Standardised test methods exist to determine whether this is the case.

These test methods provide information on the concentrations below which no adverse effects on health or the environment are to be expected. In combination with data on possible exposure levels, this information makes it possible to determine which substances can be used safely, which substances require safeguards to ensure that they can be used safely and which substances should be avoided because it is impossible to ensure that they can be used safely in practice.

Apart from the protection they offer to health, it is important that such test methods should use as few experimental animals as possible and that they are proportionate.

The OECD (Organisation for Economic Cooperation and Development) approved a new test in 2011. This test, No. 443, known as the Extended One-Generation Reproductive Toxicity Study* (EOGRTS), can be used to replace the existing test No. 416, the Two-Generation Reproduction Toxicity Study** (TGRTS).

An advantage of the new test is that the number of experimental animals required is in principle appreciably less than that needed previously (about half). In addition, the test can provide more relevant information on certain aspects.

However, use of the EOGRTS is still under discussion in the EU as part of the evaluation of proposals for new tests within the framework of the European regulation on the registration, evaluation, authorisation and restriction of chemical substances (REACH). REACH imposes strict requirements concerning the performance of animal experiments. Producers and importers of chemical substances are responsible for submitting a test proposal for the substance in question. Each proposal is scrutinised by the European Chemicals Agency ECHA, which then submits a draft decision based on the proposal to the Member States.

ECHA and some Member States still prefer use of the TGRTS. One of their reasons for this is the fear that application of the EOGRTS and hence the failure to perform tests on second generation offspring in all cases might cause valuable information about health risks to be overlooked.

In order to determine whether the new test really can serve as a replacement for the old one, it is of great importance that the EOGRTS offers at least the same level of health protection as the test used so far, in addition to the above-mentioned benefits.

In this context, I would like to ask the Health Council the following questions:

1. Does the EOGRTS, in the opinion of the Health Council, offer at least the same level of health protection as the TGRTS?
2. Is the Health Council aware of any other benefits or drawbacks of the EOGRTS compared with the existing test or tests?
3. Can the Health Council recommend use of the EOGRTS as an effective measure for ensuring the safe use of chemical substances?

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* http://www.oecd-ilibrary.org/environment/test-no-443-extended-one-generation-reproductive-toxicitystudy_9789264122550-en

** http://www.oecd-ilibrary.org/environment/test-no-416-two-generation-reproduction-toxicity_9789264070868-en

The necessary administrative steps have already been taken to prepare for your activities in this matter. In order to be able to play an effective role in the European debate on this test, I would appreciate it if you could let me have your answers to the above questions by 1 December 2012.

Yours sincerely,
The State Secretary for Infrastructure and the Environment
(signed)
Joop Atsma

Annex **B**

The Committee

- Prof. M van den Berg, *Chairman*
  Professor of Toxicology, Institute for Risk Assessment Sciences (IRAS), Utrecht
- Prof. J. de Boer
  Professor of Environmental Chemistry and Toxicology, VU University Amsterdam
- Dr. M.M.L. Dingemans
  Neurotoxicologist, IRAS, Utrecht
- Dr. D.M.G. de Groot
  Neurotoxicologist; TNO innovation for lift, Zeist
- Prof. D. Lindhout
  Professor of Medical Genetics, Utrecht University
- Prof. H. van Loveren
  Professor of Immunotoxicology, Maastricht University and National Institute of Public Health and the Environment, Bilthoven
- Prof. A.H. Piersma
  Professor of Reproductive Toxicology, Utrecht University, Utrecht, and National Institute for Public Health and the Environment, Bilthoven
- Prof. P.J.J. Sauer
  Professor of Paediatrics, University Medical Center Groningen
- Prof. F.J. van Schooten
  Professor of Genetic Toxicology, University Medical Center Maastricht

- Dr. H.E.K. de Walle
  Epidemiologist, European Surveillance of Congenital Anomalies (Eurocat), Groningen
- Prof. N. Weisglas-Kuperus
  Paediatrician, Division of Neonatology, Department of Paediatrics, Erasmus Medical Center-Sophia Children's Hospital, Rotterdam
- H.A. Meijer, *observer*
  Ministry of Infrastructure and the Environment, The Hague
- J.W. Dogger, *scientific secretary*
  Health Council of the Netherlands, The Hague

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the chairperson and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.