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**INTEGRATED RISK ASSESSMENT: NONYLPHENOL  
CASE STUDY**

**REPORT PREPARED FOR THE WHO/UNEP/ILO INTERNATIONAL PROGRAMME  
ON CHEMICAL SAFETY**

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## **INTEGRATED RISK ASSESSMENT: NONYLPHENOL CASE STUDY**

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<b>1.</b>	<b>INTRODUCTION.....</b>	<b>5</b>
<b>1.1</b>	<b>BACKGROUND.....</b>	<b>5</b>
<b>1.2</b>	<b>STRATEGIC APPROACH FOR DEVELOPMENT OF CASE STUDY.....</b>	<b>6</b>
<b>2.</b>	<b>INTRODUCTION TO NONYLPHENOL.....</b>	<b>9</b>
<b>3.</b>	<b>INTEGRATED RISK ASSESSMENT OF NONYLPHENOL.....</b>	<b>12</b>
3.1	PROBLEM FORMULATION PHASE.....	12
3.1.1	<i>Introduction</i> .....	12
3.1.2	<i>Potential benefits of integration Environmental Risk Assessment (ERA) and Human Health Risk Assessment (HHRA) knowledge</i> .....	12
3.1.2.1	Assessment questions.....	12
	Potential Integration benefits.....	12
3.1.2.2	Impetus for the assessment.....	12
3.1.2.3	Assessment Endpoints.....	13
	Potential Integration benefits.....	13
3.1.2.4	Conceptual Model.....	13
	Potential Integration benefits.....	13
3.1.2.5	Integration of the Analysis Plan.....	14
	Potential Integration benefits.....	14
3.1.3	<i>Integrated Risk Assessment</i> .....	15
	<i>Conclusions based on integration in the problem formulation phase</i> .....	17
3.2	EMISSION SOURCES, ENVIRONMENTAL CONCENTRATIONS AND EXPOSURE ESTIMATES.....	18
3.2.2	<i>Introduction</i> .....	18
3.2.3	<i>Potential benefits from integration of ERA and HHRA knowledge</i> .....	18
3.2.4	<i>Risk Assessment Data</i> .....	19
3.2.4.1	EU-RAR: General Data.....	19
3.2.4.2	EU-RAR: Environmental Concentration/ Data (Source EU-Risk Assessment Report, 2001).....	19
	Conclusions about predicted environmental concentrations.....	20
3.2.4.3	EU-RAR: Ecological Data (Source: EU-RAR, 2001).....	21
3.2.4.4	EU-RAR: Human Health Data (Source: EU-RAR, 2001).....	21
3.2.4.5	Other Data (Source: Environment Canada, 2000).....	22
3.2.5	<i>Conclusions on benefits of integration</i> .....	23
3.3	TOXICOKINETICS.....	24
3.3.1	<i>Introduction</i> .....	24
3.3.3	<i>Risk Assessment Data (Source: EU-RAR, 2001)</i> .....	24
3.3.3.1	<i>Environmental Data</i> .....	24
3.3.3.2	EU-RAR: Human Health Data.....	25
3.3.3.3	Other Data.....	25
	(Source: CAN-EPA, 2001).....	25
3.4	ESTROGENIC EFFECTS.....	26
3.4.1	<i>Introduction</i> .....	26
3.4.2	<i>Potential benefits by integration ERA and HHRA knowledge</i> .....	26
3.4.3	<i>Risk Assessment Data</i> .....	28
3.4.3.1	EU-RAR: (Source: EU-RAR, 2002).....	28
	In vitro data on ecological endocrine effects.....	28
	In vivo data on ecological endocrine effects.....	28
	Summary of ecological endocrine effects.....	28
3.4.3.2	EU-RAR: Human Health Data (Source: EU-RAR, 2002).....	28
	In vitro data on human endocrine effects.....	28
	In vivo data on human endocrine effects – EU-RAR.....	29
	Summary of human endocrine effects – EU-RAR.....	29
3.4.3.3	Other Data (Source: Environment Canada, 2000).....	30
	In vitro data on ecological endocrine effects.....	30

Summary of ecological endocrine effects .....	31
In vitro data on human endocrine effects .....	31
Summary of human endocrine effects .....	31
3.4.4 <i>Conclusions on benefits of integration</i> .....	33
Conclusions missed by not integrating.....	33
3.5 RISK ASSESSMENT FOR WILDLIFE.....	37
3.5.1 <i>Introduction</i> .....	37
3.5.1.1 Measuring NOECs for wildlife .....	37
3.5.1.2 Extrapolating rat NOAELs to NOAEL for humans .....	37
3.5.1.3 Extrapolating rat NOAEL to NOECs for wildlife.....	37
3.5.2 <i>Potential benefits by integration ERA and HHRA knowledge</i> .....	38
3.5.3 <i>Risk Assessment Data (Source: EU-RAR, 2002)</i> .....	38
3.5.3.1 Environmental Data .....	38
3.5.3.2 Human Health Data.....	38
3.5.4 <i>Conclusions on benefits of integration</i> .....	38
Differences between conclusions of the EU and Canada .....	39
Opportunities missed in the EU-RAR.....	39
4.2 EVALUATION OF THE SCIENTIFIC BENEFITS AND DRAWBACKS OF THIS NONYLPHENOL IRA .....	46
4.3 COSTS AND BENEFITS IN ECONOMICS TERMS.....	48
4.4 EVALUATION OF THE IPCS FRAMEWORK .....	50
<b>5. REFERENCES.....</b>	<b>51</b>
<b>6. APPENDICES .....</b>	<b>53</b>
6.1 APPENDIX 1: <i>IN VIVO</i> DATA ON ECOLOGICAL ENDOCRINE EFFECTS (SOURCE: EU-RAR, 2002) .....	53
6.2 APPENDIX 2: <i>IN VIVO</i> DATA ON HUMAN ENDOCRINE EFFECTS (SOURCE: EU-RAR, 2002).....	57

# 1. INTRODUCTION

## 1.1 Background

The International Programme on Chemical Safety (IPCS) was initiated in 1980 as a collaborative programme of the United Nations Environment Programme (UNEP), the International Labour Organisation (ILO), and the World Health Organization (WHO). One of the major objectives of the IPCS is to develop and promote the use of improved methodologies for assessing the risks of chemical exposures on humans and the environment. For historical and practical reasons, human health and environmental risk assessment methodologies have generally developed independently. However, there is a need for an integrated, holistic approach to risk assessment that addresses real life situations of multi-chemical, multimedia, multi-route, and multi-species exposures. In response to this need, the IPCS (in collaboration with the US Environmental Protection Agency (US EPA), the European Commission (EC), and other international and national organizations) initiated activities to develop and promote the integration of assessment approaches to evaluate human health and ecological risks.

The term “integration” can have many meanings, and several opportunities exist within risk assessment generically for integration. In the IPCS project, **integrated risk assessment was defined as a science-based approach that combines the process of risk estimation for humans, biota, and natural resources in one assessment**. The overall goal of this project was to promote international understanding and acceptance of the integrated risk assessment process. Three specific objectives were identified to meet this goal: 1) enhance understanding of the benefits of integration, 2) identify and understand obstacles to integration, and 3) engage key scientific organizations to promote discussion of an integrated approach to risk assessment. To implement these objectives, IPCS in collaboration with a working group of international scientific experts developed a generic framework (see Figure 1.) to demonstrate and communicate how an integrated risk assessment could be conducted (WHO, 2001, 2001a; Suter et al., 2003; Munns et al., 2003a). In addition, four separate case studies were developed to demonstrate the use of the framework and to highlight the benefits of using an integrated approach (Ross and Birnbaum, 2003; Hansen et al., 2003; Sekizawa et al., 2003; Vermeire et al., 2003). The framework and case studies were evaluated at an international workshop in April 2001 (WHO, 2001, 2001b). Workshop participants identified a) a number of opportunities to integrate the risk assessment process; b) benefits and obstacles to using an integrated approach; and c) research recommendations to improve and facilitate integrated approaches (Munns et al., 2003b).

One recommendation of the April 2004 workshop was to conduct an integrated risk assessment on a specific chemical to demonstrate the practical applications, benefits, and obstacles to using an integrated approach when compared to independently conducted assessments for human health and the environment. The chemical, nonylphenol (NP), was chosen for this “demonstration integrated risk assessment.” IPCS contracted with experts from the Institute of Risk Assessment, University of Utrecht, The Netherlands (in collaboration with

the Institute of Public Health and the Environment, Bilthoven, The Netherlands) to prepare the Nonylphenol case study using the IPCS generic framework for conducting integrated risk assessment.

This report summarizes the results of that effort. We hope that these collaborative efforts of the IPCS will help to establish the foundation for internationally accepted guidance for integration of risk assessment.

The draft Nonylphenol case study and related IPCS activities on integrated risk assessment were presented at the 10<sup>th</sup> International Congress of Toxicology held in Tampere, Finland, July 2004 (Munns et al., 2004; Suter et al., 2004; Sekizawa et al., 2004).

## 1.2 Strategic Approach For Development Of Case Study

The major objectives of this project was to compare a “demonstration” integrated risk assessment of Nonylphenol (NP) using the IPCS generic framework with data from independently conducted assessment on the environmental and human health effects of NP. This demonstration case study should build from the data used to conduct independent (non-integrated) assessments of the human and environmental risks of exposure to Nonylphenol. The following existing risk assessment reports on Nonylphenol were identified following an extensive literature search.

Reports with an ecological risk assessment:

- US EPA (1996) RM-1 document for para-nonylphenol.
- US EPA (2003) Ambient Aquatic Life Water Quality Criteria for Nonylphenol – Draft.

Reports with a human health risk assessment:

- None

Reports with both ecological and human health risk assessment:

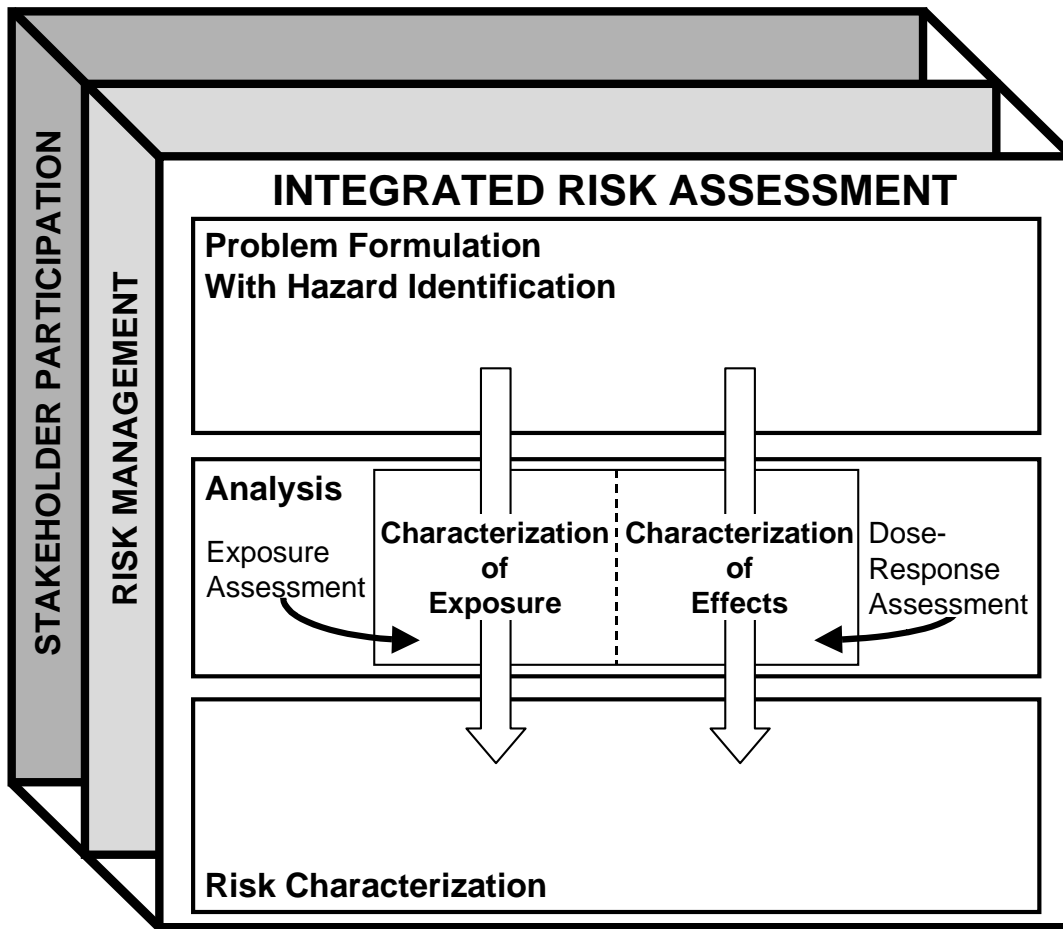
- Environment Canada (2000), Priority Substances List Assessment Report, Nonylphenol and its ethoxylates.
- EC (2001), EU-RAR on 4-nonylphenol (branched) and Nonylphenol.

Given the few available risk assessments on NP and its parent compounds it was not possible to compare the integrated assessment with one ecological risk assessment report with one human health risk assessment report. Since the EU assessment was already partly integrated and also the most recent report, it was used for this project as the major source of information (supplemented with data from the other available assessment reports). Given the limited time-frame of this project, it was not feasible to re-evaluate the primary literature. The EU approach is based on the standard approach of problem formulation, dose-response assessment, exposure assessment and risk characterization. Due to the lack of independent human health and ecological risk assessment documentation, an evaluation of the benefits and drawbacks of IRA relative to the independent human health risk assessments and ecological risk assessments was

strictly speaking not possible. Instead, the used information was classified as ecological or human data. Conclusions resulting from these data were then integrated to form evaluations of the benefits of integration. If these integrated conclusions enhanced or improved the risk assessment, the process leading to these integrated conclusions as well as the conclusions themselves were listed as benefits of integration. Potential benefits per area of integration were searched in the following benefit categories: (1) coherent expression of assessment results, (2) interdependence of the results, (3) identification of sentinel organisms, (4) enhanced scientific quality of the assessment result and (5) efficiency in using and generating data. These categories were derived from the IPCS generic framework for integrated risk assessment which is illustrated in Figure 1 (HERA, 2003).

At the start of this project a selection was made of the most promising areas for integration. “Potential areas of integration” are processes or steps within a risk assessment that use either ecological data or human health data to generate a result, but could potentially benefit from using both (i.e., areas where ecological and human data could supplement each other and generate improved or new results). Several parts of the risk assessments of Nonylphenol did not qualify for this, either because data were too limited or because integration was not relevant. The following potential areas of integration were selected (1) problem formulation, (2) sources of emission, environmental concentrations and exposure estimates, (3) toxic kinetics, (4) estrogenic effects and (5) risk assessment for wildlife.

In Section 3, the relevance of each area of integration is briefly explained. The generic benefits of integration in that area are described along with the conclusions on the specific benefits of integration in each for the chemical Nonylphenol. The data used to derive these conclusions are also summarized. For each potential area of integration, summaries are provided for: a) the benefits already apparent in the EU-RAR; b) additional benefits gained by further integration and, in some cases; c) conclusions missed by not integrating.



**Figure 1.** A framework for integrated human health and ecological risk assessment (modified from US EPA 1998). Risk assessors, risk managers, and stakeholders perform parallel activities which may interact at various stages.

## 2. INTRODUCTION TO NONYLPHENOL

This summary is based on the information in the EU risk assessment of nonylphenol.

Nonylphenol is used primarily as a building block for the monomers used in the production of resins and polymers. The data indicates that Nonylphenol is not used as a free additive in resins, plastics or stabilizers.

Of the 77,505 tons of nonylphenol (NP) produced in 1997 in Europe, 81 percent was produced continuously and 19 percent in batch production (four companies produce most of the Nonylphenol). Import exceeded export for an additional 5,000 tons in 1997. Nonylphenol is produced in Europe in three ways:

1. Phenol and mixed nonenes are reacted in the presence of a catalyst in a batch process. The catalyst used is montmorillonite clay/fulcat and phosphoric acid.
2. Phenol and mixed nonenes are reacted in the presence of a sulfonated ion exchange resin in a batch process.
3. Phenol and mixed nonenes are reacted in the presence of a fixed bed ion exchange resin in a continuous process.

Sixty percent of the NP is used for the production of nonylphenol ethoxylates, 37 percent for the production of resins, plastics, stabilizers, etc. and the remaining 3 percent for the production of phenolic oximes. All nonylphenol ethoxylates are produced from NP and ethylene oxide in batch processes. The length of the ethoxylate chain is varied by controlling the ratio of nonylphenol to ethylene oxide or by the reaction time. One company in the EU produces phenolic oximes from NP and exports all phenolic oximes out of Europe. Phenolic oximes are used as a reagent for the extraction and purification of copper from ore. The nonylphenol ethoxylates are functionally used as cleaning and washing agents, surface-active agents and foaming agents. The industry uses NP for industrial and institutional cleaning, textile auxiliaries, leather auxiliaries, emulsion polymerization, agricultural pesticides and paint production.

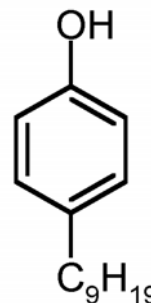


Figure 2: The molecular structure of nonylphenol.

The C<sub>9</sub>H<sub>19</sub> group can be a straight chain or be shorter and branched.

NP melts at 265 Kelvin, boils around 563 to 575 Kelvin. NP has a relative density of around 0.95 at 293 Kelvin and vapor pressure of 0.3 Pa at 298 Kelvin. Due to a low Henry's Law Constant and a low vapor pressure partitioning to air is low. NP is poorly soluble; solubility is around 6 mg/L at 293 Kelvin. NP has a pKa of 10 and will be in undissociated form in environmental water. NP has an n-octanol-partitioning coefficient (Log K<sub>ow</sub>) of around 4.48 (From EU-RAR).

Nonylphenol ethoxylate containing products used in the public domain include: non-agricultural pesticides, cosmetics, cleaning products and office products such as correction fluids and inks. EU member states and industry have agreed to phase out nonylphenol ethoxylates in all detergent applications by the year 2000. As a result, the usage of NP is expected to drop in the future.

Small emissions of NP may occur during production, and escape to air or surface water. However, most wastes are first passed through a wastewater treatment plant. Most of the NP containing compounds, such as nonylphenol (poly)ethoxylates, is then aerobically degraded by bacteria into shorter nonylphenol ethoxylates or nonylphenol. In the wastewater treatment plant, it is estimated that half of the NP adsorbs on particles. These particles then stay in the wastewater treatment plant as sludge or are emitted into the aquatic phase and settle on the sediment. For risk assessment calculations it was assumed in the EU RAR that all NP containing compounds in sludge are degraded into the more estrogenic NP. As the majority of the emissions of NP end up in the water compartment, the physico-chemical properties become relevant (Figure 2). NP is poorly soluble and unlikely to evaporate from the water. It remains in the aquatic phase and partitions to the sediment and biota such as fish. In the anaerobic sediment NP and its parent compounds (e.g. NP (poly)ethoxylates) are more resistant to degradation. Eventually, bacteria can mineralize NP. In the River Aire in England in 1995, downstream of the emission sites of textile processing industry, locally high concentrations of NP were found; up to 53 µg/L freely dissolved nonylphenol and up to 180 µg/L when including particles. Background concentrations on average in rivers in Germany in 1989 measured 0.038 µg/L, with peaks up to 1.3 µg/L. The Tees estuary in England receives water from heavily industrialized areas. In 1995 this estuary contained up to 3.1 µg/L freely dissolved nonylphenol and up to 5.2 µg/L of NP when including particles. NP has also been detected in rivers and lakes in Japan, the USA, and other countries.

Concern over the widespread usage of Nonylphenol has been increasing because of its toxicity to both marine and freshwater species, and its ability to induce estrogenic responses. The estrogenic effect of nonylphenol on fish and daphnids has been studied by a number of authors. Generally the work shows that nonylphenol and nonylphenol ethoxylates do exhibit estrogenic activity. For nonylphenol ethoxylates the activity was found to increase with decreasing chain length, with nonylphenol showing the greatest activity. Most of the tests indicate that estrogenic effects may start to occur at around 10-20 µg/L. Nonylphenol and some of its degradation products have been shown to have estrogenic activity in a number of *in vitro* (yeast, MCF-7 cells, ZR-75 cells) and *in vivo* assays (with rats and mice). The potency of this estrogenic activity in these assays ranged from 3 to 6 orders of magnitude less than that of estradiol. The effects of nonylphenol on fertility and reproductive performance have been investigated in a number of studies, but results are equivocal.

## General Conclusions on the effects of NP in the EU-RAR (2001)

In the EU risk assessment report on nonylphenol of 2001 a No Observable Effects Concentration (NOEC) of 0.33 µg/L was derived, based on the endocrine disruptive potential of NP on fresh water fish. The PNEC for sediment of 0.039 mg/kg was derived using an equilibrium partitioning method. The conclusion of the EU RAR was that calculated background concentrations of NP are of concern and further measuring and testing is needed. In addition, there are many types of industries that cause locally high concentrations of NP and risk-limiting measures would be appropriate. Secondary poisoning on locally polluted sites is possible. The terrestrial and atmospheric compartments are not likely to be the source of meaningful amounts of exposure of organisms in the environment.

The calculated oral No Observable Adverse Effects Level (NOAEL) for repeated dose for humans was 15 mg/kg/day based on the endocrine disruptive effects in rats. Data from modeling suggest that there are concerns for human health with respect to local exposure, based on low margins between modeled exposures and the N(L)OAELs for repeated dose and reproductive toxicity. Living in the locality of a textile factory increases exposure by 4.42 mg/kg/day. Exposure via occupational paint usage (approximately 2 mg/kg/day) and industry sectors of manufacturing or using NP as intermediate should be reduced. In the worst-case scenario the total exposure would be approximately 6.4 mg/kg/day. The main routes of calculated exposure of humans to background pollution from the environment are from plant roots (70 to 80%) and fish (1 to 29%). Concern for mutagenicity and carcinogenicity is low. Exposure via consumer products is negligible. The calculated dose received from background pollution from the environment on a daily basis is  $5.13 \times 10^{-3}$  mg/kg/day, which is below levels of concern.

Nonylphenol is mostly an environmental health issue for aquatic organisms. Plants are exposed to NP from sludge. Terrestrial organisms are exposed via eating plant roots or exposed via secondary poisoning. There is a measure of concern for the terrestrial environment near industrial sites, which is below levels of concern. The exposure of humans to nonylphenol from background concentrations is of limited concern. Only a local high exposure could affect human health.

### 3. INTEGRATED RISK ASSESSMENT OF NONYLPHENOL

#### 3.1 Problem Formulation Phase

##### 3.1.1 Introduction

As indicated in Figure 1, the first step in the integrated risk assessment process is problem formulation, which delineates the overall goals, objectives, scope, and activities of the integrated assessment as well as the resources available to conduct the assessment. This analysis considers whether a risk assessment is needed, and who should be involved in the assessment/risk management process. It also helps to ensure that the assessment will provide the information necessary to support the environmental decision making process (i.e. risk management). Risk managers, risk assessors and other stakeholders all bring valuable perspectives to this assessment planning.

##### 3.1.2 Potential benefits of integration Environmental Risk Assessment (ERA) and Human Health Risk Assessment (HHRA) knowledge

###### 3.1.2.1 Assessment questions

Assessment questions are those that define the goals, breadth, and focus of the assessment.

###### *Potential Integration benefits*

1. Issues that are critical for both humans and the environment are more easily identified. Integration would greatly increase the chance of serendipitous recognition of problems for which evidence in any one sector is limited, but when all data are considered together, cause for concern may become evident.
2. Assessment of risks to humans is strengthened through evaluation of risks to other organisms that influence human health and welfare.
3. There will be greater consistency in the spatial and temporal scope (e.g., with regard to the information and processes used).
4. Data and knowledge gaps are identified at an early stage.

###### 3.1.2.2 Impetus for the assessment

The impetus for the EU assessment was the requirement that all industrial chemicals be assessed for their risks to human health and the environment. The impetus for this integrated assessment was the desire of the WHO/IPCS to explore the benefits of integrated risk assessment by performing a demonstration case study.

### ***3.1.2.3 Assessment Endpoints***

Changes in the health of humans, ecosystems, or selected species (chosen to represent an ecosystem) must be quantified. Health is a qualitative endpoint but must be expressed in a measurable way. For example, endpoints such as the production of vitellogenin in male fish; mutagenicity skin irritation in rats can be used to quantify the effect of the stressor on these organisms or systems. More potential endpoints are listed in Table 2.

#### ***Potential Integration benefits***

1. Susceptible endpoints in animals or ecosystems could indicate unidentified endpoints in humans (the reverse is also possible, but is more unlikely).
2. Knowledge on the fate of the compound and the target organisms can be used to predict potential adverse effects on the health of species within an ecosystem. Respectively, knowledge on the fate of the compound and the target organs can be used to predict potential adverse health effects on the health of a species due to organ toxicity.
3. Ecologists and human health assessors can discuss the relevance of all chosen endpoints.

### ***3.1.2.4 Conceptual Model***

The core of an integrated risk assessment is the conceptual model. The conceptual model is the condensation and formulation of how the assessors (and interested parties) think the stressor reaches and distributes over the environment and affects organisms in the environment (see Figure 3 for the EU conceptual model). Ecologists and human risk assessors should work together to evaluate the common routes of exposure of the stressor in order to enhance each other's view and understanding of the stressor's behavior. The relation between emission of the stressor and endpoints measured must be well characterized.

#### ***Potential Integration benefits***

1. Both human and ecological risk assessors scrutinize the quality of information on the common pathways of emission, distribution, and exposure.
2. There will be better consensus between ecologists and human health assessors on the fate of the stressor.
3. Indirect exposure pathways via the environment to the humans are more likely to be identified since humans are modeled as just one more receiving species in the web of exposure pathways.
4. When relevant and possible, multiple sources of exposure such as consumer products, emissions, and natural emissions are incorporated into the model.
5. Only one conceptual model is produced; the ecological conceptual model and the human health conceptual model are one and are based on the same assumptions and data.

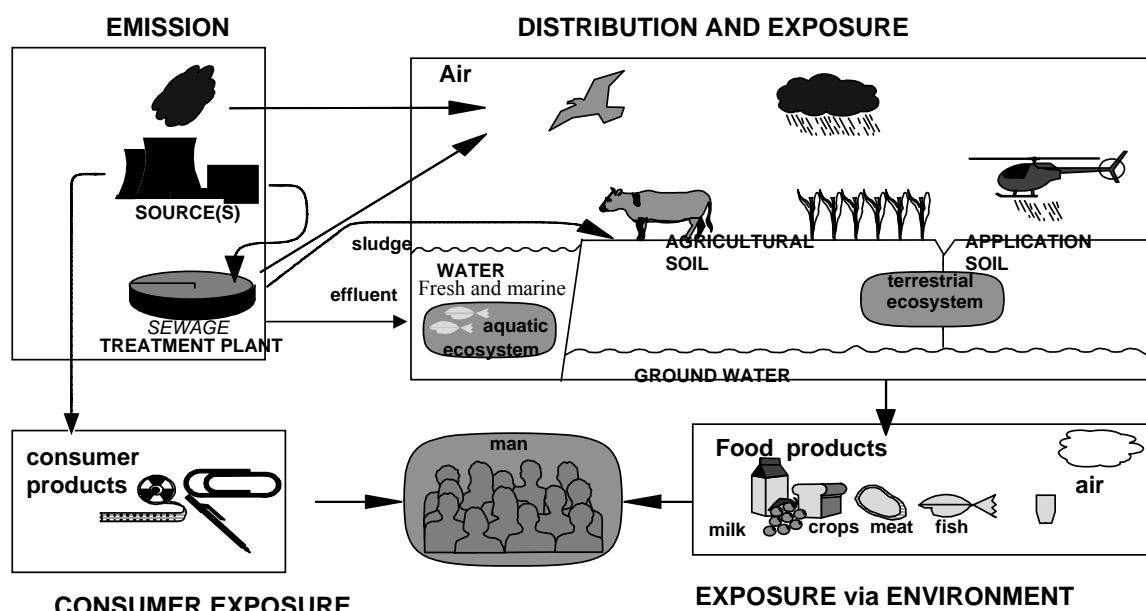


Figure 3: This figure is the summary of a non-compound specific conceptual model as used in the EU approach. The routes of exposure to the target organisms are visualized (see Table 1). Endpoints within these target organisms are defined as summarized in Table 2.

### 3.1.2.5 Integration of the Analysis Plan

In this step, the methods used to ensure the quality of the risk assessment are produced and discussed. This includes guidelines on how to select data, how to assess the quality of data, how to generate and analyze data, and how to cope with data incompleteness. The selection of acceptable computer models (if they are needed) is discussed along with a description of how results will be presented.

#### Potential Integration benefits

1. Enhanced efficiency and reduced cost since data for only one transport and fate model is needed. Emission data, physicochemical properties, fate and degradation data are only needed once. Ecological and human exposure models use the same data input. By integrating, decisions are based on more data and the available data is used more extensively.
2. Better identification of key research needs: (e.g., where do ecological and human health risk assessors lack information)? By using the same data on physicochemical properties, human and ecological health assessors can target shared research needs and transport of the stressor.
3. Ecological results and human health results adhere to the same data quality restrictions.
4. Ecological results and human health results are presented in the same terminology and if possible use the same method to express the risk and the uncertainty. This facilitates comparison between the ecological results and the human health results.
5. If the assessment will be iterated, an integrated approach might foster the development of parallel tiers, which use common data and models.

### 3.1.3 Integrated Risk Assessment

In the EU Risk Assessment framework for new and existing chemicals and biocides the stage of problem formulation is addressed, harmonized and laid down in advance of the preparation of Technical Guidance Documents (TGD) (EC, 2003). The level of integration therefore is determined largely by this general guidance and individual risk assessments for specific chemicals (such as the one for Nonylphenol) do not have an additional problem formulation stage. In some cases, the problem formulation stage results in a proposal for a more targeted risk assessment, but this has not been done for nonylphenol.

Risk assessment according to the EU-TGD is carried out in a stepwise procedure encompassing the following stages:

1. Exposure assessment: estimation of the concentrations/doses to which human populations or environmental compartments are or may be exposed.
2. Effects assessment, comprised of
  - a. hazard identification: identification of the adverse effects which may be caused by the substance
  - b. dose-response assessment: estimation of the relationship between the level of exposure to a substance (dose, concentration) and the incidence and severity of an effect.
3. Risk characterization: estimation of the incidence and severity of the adverse effects likely to occur in a human population or environmental compartment due to actual or predicted exposure to a substance.

At the risk characterization stage, this procedure will result in a quantitative comparison of the outcome of the exposure assessment and that of the effects assessment. For new and existing substances this will be a PEC/PNEC (i.e., Predicted Environmental Concentration versus a Predicted No-Effect Concentration) for environmental compartments, and a MOS (i.e., Margin of Safety), or the ratio of the estimated no-effect or effect level parameter to the estimated exposure level for human sub-populations. The risk characterization for biocides is performed by comparing the exposure to the AOEL (Acceptable Operator Exposure Level), a health based limit value. These PEC/PNEC and MOS ratios should be seen as surrogate parameters for risk characterization as they do not quantify the "incidence and severity" of adverse effects. The ratios are used as indicators for the likelihood of the occurrence of adverse effects, since a better method for a more quantitative risk characterization with general applicability is not available at the moment.

The human sub-populations and ecological systems and populations that are considered to be protection goals in the European Union System for the Evaluation of Substances (EUSES) are shown in Table 1. Examples of effects of the stressor on the health of the organisms are mentioned in Table 2.

The risk assessment for man aims at a level of protection, expressed in the MOS for new and existing substances or MOE and AOEL/exposure-ratio (biocides). These values indicate that the likelihood for adverse effects occurring is “of low concern”, taking into account the nature of the potentially exposed population (including sensitive groups); the nature and severity of the effect(s); and the uncertainties involved. In the environmental risk assessment it is assumed that ecosystem sensitivity depends on the most sensitive species and that protection of the ecosystem structure also protects community function. The PNEC derived for each ecosystem is regarded as a concentration below which an unacceptable effect will most likely not occur.

Risk assessment using the EUSES departs from a screening level approach in which so-called generic exposure scenarios are applied (see Figure 3). In the environmental risk assessment, it is assumed that substances are emitted in a standard environment with predefined environmental characteristics. No measured data are used at this level. The risk assessment covers the whole life cycle of substances as well as their fate in all environmental compartments. Four spatial scales (local, regional, continental, and global) and two time scales (acute, chronic) are distinguished. In the risk assessment for workers and consumers, again generic exposure models are applied initially, covering a wide range of applications. The resulting screening-level risk assessment is in principle valid for all EU countries, as required by the relevant EU regulations.

**Table 1 Human target populations and ecological target systems and populations in EU-RA.**

<p>Human populations:</p> <ul style="list-style-type: none"> <li>• workers</li> <li>• consumers</li> <li>• non-professional users of biocides</li> <li>• man exposed via the environment</li> </ul> <p>Ecological systems and populations:</p> <ul style="list-style-type: none"> <li>• micro-organisms in sewage treatment systems</li> <li>• aquatic ecosystem*</li> <li>• terrestrial ecosystem</li> <li>• sediment ecosystem*</li> <li>• (top) predators*</li> </ul> <p>* Fresh and marine ecosystems</p>
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**Table 2 Human endpoints and ecological endpoints in EU-RA.**

<p>Human Endpoints:</p> <ul style="list-style-type: none"> <li>• workers</li> <li>• consumers</li> <li>• non-professional users of biocides</li> <li>• man exposed via the environment</li> <li>• acute toxicity</li> <li>• irritation</li> <li>• corrosivity</li> <li>• sensitization</li> <li>• repeated dose toxicity</li> <li>• mutagenicity</li> <li>• carcinogenicity</li> <li>• toxicity for reproduction</li> </ul> <p>Ecological endpoints:</p> <ul style="list-style-type: none"> <li>• survival</li> <li>• toxicity for reproduction</li> <li>• (cell) growth</li> </ul>
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The EU exposure assessment aims at a “reasonable worst-case” scenario by applying unfavorable, but not unrealistic, standard exposure scenarios and, as much as possible, mean, median or typical parameter values. If the outcome of the reasonable worst-case risk characterization indicates that the substance is “not of concern”, the risk assessment for that substance can be stopped with regard to the life cycle stage/effect/population considered. If, in contrast, the outcome is that the substance is “of concern”, the assessment must, if possible, be refined by adapting any default parameter value for which this is considered necessary. These may include the replacement of intermediate results by: a) the results of other models judged to be more suitable for the substance under investigation; and b) the use of more reliable and representative measured data.

In the case of the EU risk assessment for Nonylphenol, further integration in the problem formulation stage could have taken place with regard to recognition of the mode of action of the chemical as an estrogen.

### **Conclusions based on integration in the problem formulation phase**

As pointed out above, integration in the problem formulation for nonylphenol in the EU risk assessment has already taken place to a certain extent with regard to:

1. assessment questions,
2. assessment endpoints,
3. conceptual models,
4. analysis plan.

The benefits of this integration have been pointed out in subsection 3.1.2. They mostly also apply to the risk characterization of nonylphenol. Further integration in the problem formulation for nonylphenol could have taken place with regard to the more explicit recognition of the mode of action of the chemical as an estrogen receptor agonist.

Without this integration the ecological and human health risk assessments may differ with regard to:

1. spatial and temporal scales considered,
2. the degree of conservatism,
3. the terminology used,
4. the scenarios for environmental exposure,
5. highlighting important effect endpoints,
6. the default values used,
7. the evaluation of the quality of the database.

## **3.2 Emission Sources, Environmental Concentrations and Exposure Estimates**

### **3.2.2 Introduction**

The scope of this section is the integration of exposure estimation, using external emission, fate and exposure models and measured concentrations in compartments and biota. External exposure can be converted to internal exposure if enough bio-kinetic information is present.

### **3.2.3 Potential benefits from integration of ERA and HHRA knowledge**

1. Routes of emission, distribution and exposure (that were defined in the Conceptual Model Stage) are translated into model equations describing how the stressor reaches the organisms via diverse routes of exposure. Here human and ecological risk assessors can discuss which models and which input values are appropriate and choose a common approach.
2. The type of model (e.g., the fugacity transport and fate model) chosen in the conceptual model stage (which includes aspects such as advection and diffusion, partitioning, bioaccumulation and abiotic and biotic degradation) can be of the appropriate space- and time scales with regard to both the human and the environmental risk assessment.
3. Monitoring data for emissions, environmental compartments (such as air, soil surface water, groundwater and marine), biota, food, and drinking water can be shared.
4. The whole life cycle of the stressor and all possible sources of emission will be considered (such as production, processing, industrial/consumer use) to ascertain potential exposure of man and the environment.
5. Local sites with high concentrations of a chemical can cause potential adverse ecological effects with indirect effects on humans. These effects are not exemplified in an ERA. For example, this means that the effects of a massive fish extinction on human health or resources would not be estimated. Integration can solve this problem.
6. Coherent conclusions can be drawn with regard to additional analytical activities necessary for higher tier human and ecological risk assessment.
7. A common approach towards uncertainty analysis can be developed.
8. A common approach to the evaluation of data quality (completeness and relevance) will be developed.

### 3.2.4 Risk Assessment Data

#### 3.2.4.1 EU-RAR: General Data

Source: EU-RAR

Nonylphenol is produced on four locations in the EU. The total production of these four locations in 1994 was 77,505 tons. This includes the NP in NP ethoxylates. The production was for 81% continuous production and 19% batch production. About 60% of the NP is processed into NP ethoxylates. NP ethoxylates are mostly used in industrial and institutional cleaning, emulsion polymerization and textile auxiliaries. Three percent of the NP is used in the metal extraction industry as phenolic oximes for the extraction of copper. The remaining 40% of the NP is mostly used for the production of resins, plastics, stabilizers etc.

#### 3.2.4.2 EU-RAR: Environmental Concentration/ Data (Source EU-Risk Assessment Report, 2001)

In the EU, NP production and the production of its parent compounds take place at only four different sites. The air compartment does not receive meaningful amounts of NP. All remaining emission is either to the wastewater treatment plant or to the incinerator. From the wastewater treatment plant NPEO or NP goes to the surface water or as sludge to agricultural soil or authorized disposal sites. Effluent is always treated at the factories. In the waste water treatment plant model, it is assumed that 2.5% of the nonylphenol ethoxylate released to the waste water treatment plant would eventually be converted and released to surface waters as nonylphenol. Based on estimated emissions in the amount of nonylphenol released to surface water as a result of the use of nonylphenol ethoxylates is estimated as 2,690 kg/day in the continental model and 299 kg/day in the regional model.

Only a few studies have been done on the measurements of environmental concentrations in the compartments of air, soil and landfills. There are no reported measurements of nonylphenol in the atmosphere. Sludge was applied to the top 5 cm of grassland: the initial concentration of nonylphenol in the soil was 4.7 mg/kg, but this had dropped to 0.46 mg/kg dry weight after 322 days. The concentration of nonylphenol in grassland soil that had not been treated with sewage sludge was <0.02 mg/kg (dry weight). The study also looked at sludge-only landfill sites. The concentration of nonylphenol in the sludge samples ranged from 4-37 mg/kg (dry weight) for raw sewage sludge and 7-375 mg/kg (dry weight) for digested sludge. Measurements of NP in surface water are more readily available. The nonylphenol concentration in the river Main in Germany was monitored throughout the years 1989-1991. The nonylphenol concentration in the water of the Main (June 1991) was mostly 0.18 µg/L or less. More concentration data are available but concentrations in rivers in general do not exceed the 5 µg/l. In the English river Lea the total extracted amount of NP is 0.5 to 12 µg/l and the dissolved fraction of NP is 0.2 to 9.0 µg/l. Concentrations of NP in groundwater due to infiltration of river water are low. In seawater the concentration of NP only increased in estuaries that received water that carried effluent from industries. The highest concentrations were observed in the Tees estuary at 0.08 to 3.1 µg/l dissolved NP and 0.09 to 5.2 µg/l total extracted NP.

## *Conclusions about predicted environmental concentrations*

### Soil:

PEC <sub>continental</sub> <sub>agri, soil</sub>	= 0.0271 mg/kg wet weight
PEC <sub>continental</sub> <sub>nat, soil</sub>	= $2.39 \times 10^{-6}$ mg/kg wet weight
PEC <sub>continental</sub> <sub>pore water</sub>	= $2.86 \times 10^{-4}$ mg/kg wet weight
PEC <sub>regional</sub> <sub>agri, soil</sub>	= 0.265 mg/kg wet weight
PEC <sub>regional</sub> <sub>nat, soil</sub>	= $1.44 \times 10^{-5}$ mg/kg wet weight
PEC <sub>regional</sub> <sub>pore water</sub>	= $2.8 \times 10^{-3}$ mg/kg wet weight

### Air:

PEC <sub>continental</sub> <sub>air</sub>	= $5.21 \times 10^{-7}$ mg/m <sup>3</sup>
PEC <sub>regional</sub> <sub>air</sub>	= $3.14 \times 10^{-6}$ mg/m <sup>3</sup>

### Surface water:

PEC <sub>regional</sub> <sub>surface water</sub>	= 0.60 µg/l
PEC <sub>continental</sub> <sub>surface water</sub>	= 0.066 µg/l
Sediment of freshwater:	
PEC <sub>regional</sub> <sub>sediment</sub>	= 103 µg/kg wet weight
PEC <sub>continental</sub> <sub>sediment</sub>	= 13.1 µg/kg wet weight

### Water concentrations:

In top water the concentration of nonylphenol was between 1.7 to 3.02 µg/l, in middle waters the concentration was between 1.3 to 1.6 µg/l and in bottom waters between 0.54 to 1.2 µg/l. These measured levels will be used in the risk characterization section.

### Surface water concentrations:

Based upon background data, concentrations of nonylphenol in surface waters would appear to be relatively low when compared to calculated levels (0.12 µg/L USA; 0.18 µg/L Glatt river; 0.01 µg/L Finnish lake water; 0.01 to 0.08 µg/L Bavarian rivers; <0.5 µg/L Hessian rivers). A background concentration of nonylphenol of 0.2 µg/L therefore appears to be a realistic level. The calculated predicted environmental regional (PEC<sub>regional</sub>) based upon default releases is 0.6 µg/L which, although higher, is of the same magnitude. The recently measured data are typical of areas where the use of ethoxylates has been controlled to some extent, but may not be representative of areas where widespread use still occurs. Therefore the calculated PEC<sub>regional</sub> will be used in the risk assessment, as this is taken as representing an area with widespread use of nonylphenol or nonylphenol ethoxylates. The estimated regional predicted environmental concentration (PEC) for surface water (0.6 µg/l) exceeds the aquatic (PNEC) of 0.33 µg/l.

### Ground water

The groundwater levels reported should be used with care as they relate to river water infiltration into groundwater.

### Sediment

A wide range of sediment concentrations is reported. As with the other data, the concentrations appear to vary widely depending upon the inputs to the receiving waters. The calculated levels were again similar to the measured levels.

### Wastewater effluent

The measured levels downstream of wastewater treatment plants receiving industrial effluents are generally lower than the PEC local calculated for specific industries. This suggests that the PEC calculations are overestimating the concentrations in receiving waters. The measured data however are not comprehensive enough to have covered receiving waters from all the different industry types which use nonylphenol or nonylphenol ethoxylates. Therefore the calculated PECs will be used in the risk characterization section despite the concerns over the assumptions made in generating the data.

### Soil with sludge

The Danish EPA reports that levels of nonylphenol in soil after sludge application are typically 0.3-1.0 mg/kg but that they can go up to 4.7 mg/kg. These measured levels are of the same order as a number of those calculated, but the PECs for some industries are much higher.

#### **3.2.4.3 EU-RAR: Ecological Data (Source: EU-RAR, 2001)**

Nonylphenol shows a high bioconcentration potential in aquatic organisms. A PNE Coral of 10 mg/kg food was derived for a secondary poisoning scenario. The concentration of nonylphenol in fish and earthworms for predators (mammals and birds) has been estimated. There are 12 lifecycle stages of NP containing products that can cause potentially harm to fish-eating or earthworm-eating organisms.

#### **3.2.4.4 EU-RAR: Human Health Data (Source: EU-RAR, 2001)**

The highest estimate for exposure of man *via* the environment (not in the vicinity of a nonylphenol plant) is provided by the regional model at  $5.31 \times 10^{-3}$  mg/kg/day. The maximum combined local intake, taking account of exposure via air, drinking water and food is 4.42 mg/kg/day (from the textile industry). The highest exposure an individual is likely to experience would occur if they apply specialty paints (2 mg/kg/day), use a pesticide product (0.35 µg/kg/day), use cosmetics (0.1 µg/kg/day) and are exposed via food packaging materials (0.2 µg/kg/day) while living in the locality of a textile factory (4.42 mg/kg/day). The maximum combined daily total exposure for an individual is approximately 6.4 mg/kg/day from the estimates provided in the EU report. However, there is considerable uncertainty in the estimated human daily intake figures; consequently the accuracy of the predictions is difficult to determine. The first cause of uncertainty results from the lack of reliable data on the quantities of nonylphenol released into the environment from actual production and various uses. Releases and hence concentrations from actual production and use sites are likely to be much lower than

the figures used here. The second cause of uncertainty concerns the assumption made in the local calculations that all of the water, air and food comes from close to a point source of release. The largest contributor to the human exposure is the intake of fish. This accounts for around 70-80% of the daily dose. The other significant contributor is the intake of plant roots (1-29%). The figures are based on predicted environmental concentrations in a local scale scenario. The calculations may be overestimates but the degree of overestimation is uncertain. A number of emission scenarios in the model are based upon default estimations. This may result in significant variations between predicted concentrations and actual environmental concentrations.

### **3.2.4.5 Other Data (Source: Environment Canada, 2000)**

A steady-state, non-equilibrium model (EQC Level III fugacity model) was run using the methods developed by Mackay (1991) and Mackay and Paterson (1991). The results of this modeling predicts that when NP is released in water, most of it is present in water (49–59%) and, to a lesser extent, sediment (41–50%), with a negligible proportion (<1%) in air and soil. When emissions are released to the soil, most of the NP would remain in the soil. When emissions are released to the air then most of the NP would be in the air. Emission to surface water is also a very realistic emission scenario. Sludge application to land is considered in the human health assessment. Soil (e.g. attached. to vegetables) eaten by humans is considered non-degraded sludge mixed with soil.

**Data on human exposure in the Canadian assessment are summarized below:**

<b><u>Route Of Exposure</u></b>	<b><u>Dose Per Route (mg/kg-bw/day)</u></b>
air	0.000016
surface water	0.00039
food packaging	0.017
meat	0.017
sludge-amended soil	0.0000025
LOEL of 12 mg/kg-bw/day for rats, (based on an oral dose for 3 generations long)	12

Human exposure was calculated based on limited data on measured human concentrations in combination with consumption information. Due to worst-case scenarios and lack of actual exposure data the calculated human exposure dose is quite uncertain. By combing data for each route of exposure, the total human exposure dose adds up to 27 mg/kg-bw/day. This is a worst case scenario using limited environmental sampling measurements. Although, the EU assessment used a model and the Canadian assessment used scenarios to calculated human exposure, the calculated worst-case exposures were in the same order of magnitude.

### **3.2.5 Conclusions on benefits of integration**

#### **Integration benefits already present in EU-RAR**

In the EU-RAR, the exposure estimates are already performed in an integrated manner and the following benefits of integration can be identified:

1. A common modeling approach is applied to the exposure estimation of nonylphenol for ecological receptors and humans leading to a coherent assessment and avoiding duplication of work. For example, the same estimated emission and background levels are used in the exposure estimation for environmental organisms and for human beings. The estimation of exposure of man via fish intake is estimated from the surface water concentration. The same concentrations also determine the risks for aquatic organisms.
2. This approach uses common space and time scales. The risk assessment for ecosystems and humans exposed via the environment is done at a local scale (around a point source) as well as at a regional level (considering all point sources together). Short-term exposure levels during emission periods are estimated as well as annual averages for long-term exposures.
3. The available monitoring data for (concentrations in environmental compartments, biota, food and feed and drinking water) are shared. This avoids the use of different data of different quality in independent assessments. It results in a common approach towards the analysis of the accuracy, specificity and relevance of these data.
4. The whole life cycle of nonylphenol and all possible sources of emission are considered in both the environmental and the human health risk assessment, stimulating a holistic view and a coherent development of risk reduction options.
5. The nonylphenol assessment shows a common approach to the evaluation of data quality, completeness, and relevance as outlined in the TGD (EC, 2003).

Coherent conclusions can be drawn with regard to additional analytical activities necessary for higher tier human and ecological risk assessment. This will promote the best use of the available resources and an integrated sampling strategy. For example, when a lack of data is identified then default worst-case or reasonable worst-case scenarios are used to make an estimate. When the PEC/PNEC ratio is close to or higher than one, the risk assessment is refined via an iteration of collection of new and/or more precise data until either the ratio is reasonably smaller or it becomes apparent that the new data confirm a higher risk estimate and risk reductive measurements are needed. Guidelines on which type of data to refine first are available in the TGD. Parameters that need refinement are selected based on sensitivity analysis. The execution of an actual monitoring program is one of the last options to gain more reliable environmental concentrations.

#### **Additional benefits of integration**

No additional benefits are identified above those already present in EU-RAR.

### **3.3 Toxicokinetics**

#### **3.3.1 Introduction**

Toxicokinetics represent the fate of a chemical within the organism. This includes (1) the uptake of NP after exposure; (2) the biotransformation of NP; (3) the tissue distribution of NP and its metabolites, and (4) the elimination of NP and its metabolites. Accumulation is a result of the complex toxicokinetics behavior in the food chain. Accumulation can lead to increased exposure at higher trophic levels.

#### **3.3.2. Potential benefits by integration ERA and HHRA knowledge**

Potential benefits of linking ERA in relation to toxicokinetics are:

1. ADME (Absorption, Distribution, Metabolism and Excretion) data and toxicokinetic models for laboratory animals and man may be extrapolated to wildlife.
2. Development and improvement of Physiologically Based Pharmacokinetic (PBPK) modeling by combining data sets of different species could improve interspecies extrapolation.
3. Identification of metabolic pathways observed in environmental biota, in laboratory mammals or humans may strengthen each other.
4. Information about elimination rates in environmental biota, mammalian test organisms or in humans lead to better predictions on the bioaccumulative potential.
5. Information about accumulation in specific biota may point to relevant uptake routes for humans (for example: if a particular chemical has limited accumulation in a certain food product there will be no need to take this route of exposure into account in the human health risk assessment. In the opposite case: chemical accumulation might occur in an unexpected manner).
6. Biomarkers developed for one species may be useful for exposure/effect assessment in other species. Biomarkers (e.g., DNA-adduct formation or the occurrence of certain metabolites in urine) could be used to demonstrate the presence of the stressor and help quantify exposure.

#### **3.3.3 Risk Assessment Data (Source: EU-RAR, 2001)**

##### **3.3.3.1 Environmental Data**

It is clear from the available data that nonylphenol bioconcentrates to a significant extent in aquatic species, with bioconcentration factors (BCFs) (on a fresh weight basis) of up to 1,300 in fish. However, this value may overestimate the BCF; more reliable values with a mean of 741 have been measured, which are of a similar order of magnitude. Bioconcentration factors of around 2000-3000 have been measured in mussels. The BCF calculated from the log Kow of 4.48, using the TGD equation, is 1,280, which agrees well with the measured values. The calculated value of 1,280 will be used in the risk assessment. Nonylphenol was detected in the

following tissues of experimental fish in descending order of concentration: bile, liver, kidney, fat, gill, heart, muscle.

### **3.3.3.2 EU-RAR: Human Health Data**

Most of the information on the toxicokinetics of nonylphenol concerns oral exposure and is based on a small number of limited rat and human studies. This is supported by data on octylphenol (an alkylphenol with a close structural relationship to NP). The available data, though sparse, does provide the basis for a general understanding of the main features of the toxicokinetic profile. Absorption from the gastrointestinal tract is initially rapid, and probably extensive. The major metabolic pathways are likely to involve glucuronide and sulphate conjugation, and there is evidence of extensive first pass metabolism of nonylphenol absorbed through the gastrointestinal tract. Because of first pass metabolism, the bioavailability of unconjugated nonylphenol is probably limited following oral exposure (at no more than 10-20% of the administered dose). Nonylphenol is distributed widely throughout the body, with the highest concentration in fat. Available data on bioaccumulation potential from both animal and human studies are inconsistent and do not allow for conclusions on the bioaccumulation potential of NP. The major routes of excretion of NP are via the faeces and urine. There are no data on the toxicokinetics of nonylphenol following inhalation exposure, but on the basis of the oral absorption data and high partition coefficient, it would be prudent to assume that significant absorption via the inhalation route can occur. Because first pass metabolism will not take place following exposure by this route, the systemic bioavailability is likely to be substantially greater than is associated with the oral route. Concerning the dermal route, *in vitro* data indicate that nonylphenol is poorly absorbed across skin, although there is some limited skin penetration.

### **3.3.3.3 Other Data**

#### **(Source: CAN-EPA, 2001)**

Nonylphenol accumulates in several species of plants and is metabolized to hydroxylated and conjugated derivatives.

#### **(Source: US-EPA, 2003)**

Nonylphenol is metabolized by cytochrome P450 enzymes in the rainbow trout. Bile was found to be the major route of excretion for both waterborne and dietary exposure of the fish.

#### **(Source: US-EPA, 2003)**

Nonylphenol bioaccumulates in aquatic organisms to low levels. In freshwater fish, lipid normalized bioconcentration factors ranged from 39 to 209. Bioaccumulation was apparently greater in saltwater organisms, where bioconcentration factors ranging from 78.75 to 2,168 were measured.

### **Conclusions on benefits of integration**

Integration benefits already present in EU-RAR: None.

## **Additional benefits of integration**

Table 3 summarizes the available information on the toxicokinetics of nonylphenol. The only additional insight gained here is that the metabolism of nonylphenol proceeds through similar pathways across species, including humans. Overall the data are rather limited for integration.

### **3.4 Estrogenic Effects**

#### **3.4.1 Introduction**

One of the reasons for a risk assessment on nonylphenol (NP) for both the EU and Canada was the demonstrated estrogenic effects of NP and its parent compounds on fish and mammals. Since direct human health data are not available, the available data on estrogenic effects in experimental animals are used to extrapolate to humans. Various endpoints have been used to measure the estrogenic effects of NP and other estrogenic chemicals (Damstra et al., 2002). For example, increased vitellogenin production can be used as an endpoint in fish to measure exposure to a high nonylphenol concentration (or another estrogenic compound). Endpoints can indicate the effect of the stressor on an ecosystem, a population or a single organism.

#### **3.4.2 Potential benefits by integration ERA and HHRA knowledge**

Benefits on the understanding of mechanism of action and endpoints:

1. Mechanisms of action can be confirmed by looking across the species barriers. Estrogen/steroid modes of action in fish and mammals can be the same, as estrogen receptors are often highly conserved in structure.
2. Opportunity to detect (common) critical pathways from target site to endpoint across species may be identified.
3. Knowledge on links between molecular events and endpoints will be improved.
4. Effect found in wildlife populations might identify new endpoints, and could assist in identifying emerging new risks for humans. This also reduces the chances of overlooking critical effects.

**Table 3: Toxicokinetics**

<b>Knowledge in this area of integration</b>	<b>Additional conclusions (based on integration)</b>
Accumulation in plants seems negligible. Accumulation in fish is of a low to moderate level.	The uptake of NP into plants seems negligible, thus concentrations in plants will be negligible. So exposure via plants is not expected to be an important route of exposure for humans. However, the data sets that support the low uptake of plants are rather limited.
Metabolism by cytochrome P450 enzymes and subsequent glucuronidation is the major pathway of NP in fish, laboratory animals and humans.	The metabolization of NP is based on the same mechanism in diverse taxa.
A major route of excretion of NP in fish was via bile. This route was not investigated in laboratory animals and humans. In laboratory animals, though, excretion of radiolabel occurred mainly via faeces, while in humans most excretion occurred via urine.	As faeces contain bile, the routes of excretion of NP in fish and laboratory animals are similar. The route of excretion via urine in humans is different.
Although several studies on toxicokinetics and metabolism are performed with mammals and major metabolites are identified, no PBPK models are applied to these toxicokinetic studies.	A comparison of kinetics in mammals and environmental biota is impossible.

Benefits for dose-effect relations:

5. Integration strengthens association of exposure-effects in one species if the same exposure-effect relation is also observed in other species.

Benefits for extrapolation from human health data to wildlife data:

6. Improved cross-species extrapolation might reduce or validate extrapolation factor values.
7. In the absence of toxicity data for predators (for example fish-eating birds, fish-eating mammals and organisms feeding on earthworms in the ecological risk assessment), toxicity data for rats or mice (generated in the framework of the human health risk assessment) can be used to evaluate the hazard to predators.
8. Effects found in wildlife populations might serve as a warning for low dose-effect in human populations. In the same reasoning: one sensitive species may serve as “early warning” for other organisms, including humans.

### 3.4.3 Risk Assessment Data

#### 3.4.3.1 EU-RAR: (Source: EU-RAR, 2002)

##### ***In vitro data on ecological endocrine effects***

Vitellogenin production by isolated hepatocytes from rainbow trout has been used as an *in vitro* test system for estrogenic activity of nonylphenol and several nonylphenol ethoxylates. Vitellogenin is a yolk protein normally produced in response to estrogen in female trout. The relative potency of nonylphenol to estradiol -17 $\beta$  was 0.0000090. The mean EC<sub>50</sub> for the test was measured at 16.15  $\mu$ M nonylphenol (3.56 mg/l). Studies have reported that nonylphenol can stimulate vitellogenin secretion, *in vitro*, at concentrations of 10<sup>-6</sup> M (0.2 mg/l) and above in hepatocytes from rainbow trout. Nonylphenol showed competitive displacement of estrogen from its receptor site in rainbow trout.

##### ***In vivo data on ecological endocrine effects***

A summary of *in vivo* data is provided in Appendix 1.

##### ***Summary of ecological endocrine effects***

The estrogenic effect of nonylphenol on fish and Daphnids has been studied by a number of authors. Generally the work shows that nonylphenol and nonylphenol ethoxylates do exhibit estrogenic activity. For nonylphenol ethoxylates the activity was found to increase with decreasing chain length, with nonylphenol showing the greatest activity. Most of the tests indicate that estrogenic effects may start to occur at around 10-20  $\mu$ g/L.

#### 3.4.3.2 EU-RAR: Human Health Data (Source: EU-RAR, 2002)\*

##### ***In vitro data on human endocrine effects***

4-Nonylphenol was one of a number of alkyl phenols tested in a yeast assay in a study which looked at the structural features important for estrogenic activity in this chemical group (Routledge and Sumpter, 1997). The assay uses a recombinant strain of yeast (*Saccharomyces cerevisiae*) which contains an estrogen-inducible expression system. In the presence of estrogens a reporter gene (Lac-Z) encoding for the enzyme  $\beta$ -galactosidase is expressed, which can be monitored by measuring a colour change reaction in the culture medium. The estrogenic activity of the test substances was expressed as a potency relative to 17 $\beta$ -estradiol by comparing the molar concentrations required to produce the same response. 17 $\beta$ -estradiol was found to be about 30,000 times more potent than nonylphenol. Tamoxifen, an estrogen antagonist known to act via the estrogen receptor, was shown to inhibit the activity of the alkyl phenols, demonstrating that the assay response was due to interaction with the estrogen receptor. The estrogenic activity of nonylphenol has also been assessed in an *in vitro* assay involving estrogen sensitive human

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\* Individual references are listed in the EU-RAR.

breast tumor MCF-7 cells containing human ER $\alpha$  (Soto *et al.*, 1991). The cells are cultured in the presence of charcoal-stripped (to remove endogenous estrogens) human serum so cell proliferation is inhibited. Substances with estrogenic activity can then overcome this inhibition. The MCF-7 cells were cultured in the presence of 17 $\beta$ -estradiol or nonylphenol at several concentrations in triplicate in multi well plates. Cell proliferation was assessed after a six-day exposure period by counting nuclei from lysed cells. Nonylphenol at a concentration of 10  $\mu$ M elicited a similar proliferative response to estradiol at a concentration of 30 pM; thus, on a molar basis the estrogenic potency of estradiol, as measured in this assay, is 3.0E6 times greater than that of nonylphenol. At concentrations of 1 and 0.1  $\mu$ M the proliferative response produced by nonylphenol was similar to that observed in vehicle treated control cultures.

In another similar *in vitro* assay, MCF-7 and ZR-75 human breast cancer cell lines were used (White *et al.*, 1994). Cells were cultured in quadruplicate in the presence of nonylphenol at concentrations ranging from 0.1 nM to 10  $\mu$ M or 17 $\beta$ -estradiol at 10 nM. No estrogenic activity was detected at nonylphenol concentration of 100 nM and less. At 1 and 10  $\mu$ M nonylphenol elicited a proliferative response which at the higher concentration was similar to that produced by estradiol. Thus, 17 $\beta$ -estradiol was 1000 times more potent than nonylphenol in this assay. In a further investigation, the ability of nonylphenol to stimulate transcriptional activity was determined in MCF-7 and chicken cell fibroblasts (CEFs) transfected with reporter gene pEREBCAT and a mouse estrogen receptor. Nonylphenol stimulated transcription at culture concentrations of 1 and 10  $\mu$ M.

To summarise the *in vitro* estrogenic data, there is evidence that nonylphenol has estrogenic activity, of 3-6 orders of magnitude less potent than estradiol.

### ***In vivo data on human endocrine effects – EU-RAR***

A summary of data on *in vivo* effects is provided in Appendix 2.

### ***Summary of human endocrine effects – EU-RAR***

No human data are available. Nonylphenol has been shown to have estrogenic activity in a number of *in vitro* (yeast, MCF-7 cells, ZR-75 cells) and *in vivo* assays (with rats and mice). The potency of this estrogenic activity in these assays ranged from 3 to 6 orders of magnitude less than that of estradiol. The effects of nonylphenol on fertility and reproductive performance have been investigated in a dietary administration in a multigeneration study in the rat. This study provided evidence that nonylphenol exposure over several generations can cause minor perturbations in the reproductive system of offspring, namely slight changes in the oestrous cycle length, the timing of vaginal opening and possibly also in ovarian weight and sperm/spermatid count. Functional changes in reproduction were not induced at the dose levels tested. The NOAEL for these changes was 15 mg/kg/day. The observed perturbations in offspring are compatible with the predictable or hypothesized effects of exogenous estrogenic activity. Evidence of testicular toxicity, seen as seminiferous tubule vacuolation, cell necrosis and a reduction in tubule diameter, was reported at exposure levels which also cause mortality in a repeated dose gavage study in rats. The LOAEL for testicular toxicity was 100 mg/kg/day. The toxicity of nonylphenol appears to be enhanced by gavage administration in comparison to

dietary administration, presumably because higher peak blood concentrations of nonylphenol are achieved by gavage.

A standard oral developmental toxicity study in the rat showed no developmental toxicity of NP. Maternal and foetal NOAELs were 75 and 300 mg/kg/day, respectively. In contrast, in a gavage study involving *in utero*, lactational and direct post-weaning exposure, there was a reduction in sperm count at 250 mg/kg/day (although it is not possible to state whether this is a developmental effect or a result of direct exposure after weaning). In an intraperitoneal study designed to investigate the effects of nonylphenol on male reproductive tract development of neonatal rats, evidence of impaired development was observed. However, this study was difficult to interpret, so that these results carry minimal weight in the overall assessment of the available data.

Overall, the results of estrogenic activity in the *in vitro* and *in vivo* assays showed minor perturbations in the reproductive system of offspring in the multigeneration study, and testicular changes in gavage studies collectively raise concerns for reproductive toxicity, possibly mediated through action on the estrogen receptor. These concerns for reproductive toxicity are addressed in the risk characterization, although there are uncertainties. The estrogenic activity assays are merely screening tests. The effects on reproduction-related parameters in the multigeneration study were marginal and there was no evidence of functional changes in reproduction; furthermore any changes that were seen occurred at exposure levels in excess of the LOAEL for repeated dose toxicity (LOAEL for renal toxicity is 15 mg/kg/day, NOAEL for reproductive changes is 15 mg/kg/day). Evidence of testicular toxicity was reported in two repeated exposure studies designed specifically to investigate the effects on this organ, but only at doses which also caused mortality. No evidence of testicular toxicity was seen in standard repeated dose studies involving dietary administration. Development was not affected in a standard rat oral developmental toxicity study. With respect to the effects on the reproductive system, a NOAEL of 15 mg/kg/day has been established in a multigeneration study and this value is used in the risk characterization.

#### **3.4.3.3 Other Data (Source: Environment Canada, 2000)\*\***

##### ***In vitro data on ecological endocrine effects***

Limited data on *in vitro* test results were available and are provided in the summary.

The relative potency of NP was  $8.9E^{-5}$  to  $2.0E^{-4}$  compared to estradiol; the relative binding affinity to  $E_2$  receptor was (Kd)  $5.0E^{-5}$  M, binding to estrogen receptor in trout was  $2.54E^{-4}$ .

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\*\* Individual references are listed in the Environment Canada report (2000).

### ***Summary of ecological endocrine effects***

Alkyl phenols (APs) and alkyl phenol ethoxylates (APEs) have been reported to cause a number of estrogenic responses in a variety of aquatic organisms. These responses occur at concentrations similar to those at which chronic effects are reported in aquatic biota. Experiments in several different *in vitro* systems have indicated similar relative potencies among NPEs. NP was found to be  $\sim 1.0E5$  times less potent than estradiol ( $E_2$ ). NP<sub>2</sub>EO and NP<sub>1</sub>EC were only slightly less potent than NP in inducing vitellogenin in trout hepatocytes. Addition of EO units to NPEs reduced the potency, such that NP<sub>9</sub>EO was an order of magnitude less potent *in vitro*. APEs bind to the estrogen receptor, resulting in the expression of several responses, including the induction of vitellogenin in both *in vitro* and *in vivo* systems. One of the functions of endogenous estrogens in fish is to stimulate the liver to produce vitellogenin, a large phospholipoprotein. It is released into the bloodstream and sequestered by developing oocytes for production of egg yolk. In maturing female fish, vitellogenin is a major constituent of blood proteins; in male fish, it is not normally present in appreciable amounts. If male fish are exposed to estrogens, however, vitellogenin can be produced at similar levels to those found in maturing females. Although the implications of the induction of vitellogenin for the reproductive function of fish are not fully understood, it has been used as a very sensitive indicator of exposure of fish to exogenous estrogens. Jobling *et al.* (1996) determined the potency of NP<sub>2</sub>EO and NP<sub>1</sub>EC to be only slightly less than that of NP in rainbow trout. Jobling *et al.* (1996) also demonstrated that NP<sub>2</sub>EO and NP<sub>1</sub>EC had similar potency for *in vivo* induction of vitellogenin in rainbow trout. The threshold for vitellogenin induction in fish is 10  $\mu\text{g/L}$  for NP in the water (Jobling *et al.*, 1996). The induction of mRNA coding for vitellogenin in rainbow trout was recently reported at 1  $\mu\text{g NP/L}$  (Fent *et al.*, 1999). The estrogenic responses of NP and NPEs appear to be at least additive (Soto *et al.*, 1994; Sumpter and Jobling, 1995) and should, therefore, be considered as a group. The threshold for expression of intersex (ova-testes) in killifish was  $<50 \mu\text{g NP/L}$  (Gray and Metcalfe, 1997). APEs also affect the growth of testes in fish, alter normal steroid metabolism and disrupt smoltification (Fairchild *et al.*, 1999). There is currently considerable debate resulting from the inconsistency in relative potency reported for estradiol receptor binding, yeast estrogen screen (YES) assay and vitellogenin induction in trout hepatocytes. Additional research is required to fully understand the potential estrogenic effects of APs and APEs on the environment. The significance of estrogenic responses to the individual or population is also not known.

#### ***In vitro data on human endocrine effects***

In *in vitro* studies, NP activated the estrogen receptor with a potency 5000–7000 times less than that of  $17\beta$ -estradiol (Routledge and Sumpter, 1996; Gaido *et al.*, 1997; Odum *et al.*, 1997). In MCF-7 human breast cancer cells, cell proliferation was stimulated by NP at concentrations between 0.1 and 10  $\mu\text{M}$  (22 and 2203  $\text{mg/L}$ ) (White *et al.*, 1994; Villalobos *et al.*, 1995; Blom *et al.*, 1998).

#### ***Summary of human endocrine effects***

In a multigeneration study in which rats were exposed to NP in the diet, the LOEL was 200 ppm in diet (equivalent to a mean dose of approximately 12-18  $\text{mg/kg-bw}$  per day in males,

16–21 mg/kg-bw per day in non-lactating females or 27-30 mg/kg-bw per day in lactating females), based on an increase in renal medullar tubular dilation and cyst formation in males in all generations (F0–F3) and in F3 females. There were also increases in gestation length and in percent abnormal sperm morphology observed in the F2 generation at this dietary level, as well as at the 650 ppm and at 2000 ppm, but these were probably not treatment-related. In both cases, the increase was small, not clearly dose-related, and within the range of control values from other generations and from historical controls. As well, these effects were not observed in other generations and the F2 control values were unusually low. No developmental effects were reported at any dietary level; however, a range of effects on endocrine-regulated endpoints, including delayed vaginal opening, was observed at 650 and 2000 ppm. In reproductive toxicity studies, histological changes in the seminiferous vesicles of the testes of rats were observed following oral exposure to 100 mg NP/kg body weight per day for 10 days. This was accompanied by compound-related mortality at doses that did not cause deaths in several other studies. Reductions in relative testis, epididymis, seminal vesicle and prostate weights were reported in rat pups exposed to 0.8 mg NP/kg-bw per day intraperitoneally in the first 15 days after birth. However, this information is not considered directly relevant to the margin of exposure, in view of the lesser relevance of this route of administration.

In a number of *in vivo* and *in vitro* studies, NP has been weakly estrogenic. NP increased uterine weight in immature or ovariectomized rats and in mice following oral administration of 50 mg/kg body weight per day and above following subcutaneous or intraperitoneal. Several other effects indicative of estrogenic activity have been observed in rats following the subcutaneous administration of NP *in vivo*, including endometrial proliferative response, and stimulation of uterine vascular permeability. An increase in cell proliferation in the mammary gland of rats exposed to 0.01 mg NP/day by subcutaneous minipump has also been reported; however, this effect was not reproducible in two subsequent studies. NP was 1000–100 000 times less potent than estradiol in stimulating estrogenic activity. In *in vitro* studies, NP activated the estrogen receptor with a potency 5000–7000 times less than that of 17 $\beta$ -estradiol. In MCF-7 human breast cancer cells, cell proliferation was stimulated by NP at concentrations between 0.1 and 10  $\mu$ M (22 and 2203 mg/L).

The potential estrogenicity of NP and NPEs has been investigated in a number of studies. NP and NP2EO activated the estrogen receptor and had some estrogenic activity *in vitro*. NP was uterotrophic or induced other effects indicative of estrogenic activity in several studies *in vivo*. However, these compounds were between 3 and 5 orders of magnitude less active in this regard than estradiol. In addition, NP was estrogenic only at relatively high dose levels; for example, effects on renal histopathology were observed at 3 times lower doses of NP than those in estrogen responsive tissues in the multigeneration study in rats (i.e., 12 vs. 50 mg/kg-bw). In addition, NPEs of longer chain lengths (4, 9 and 12) were not uterotrophic *in vivo*, and NP12EO was not estrogenic in a recombinant yeast screen assay.

Hence, while it is clear that NP and some short chain NPEs have estrogenic potential, the evidence that this is a critical effect of these substances is considered inadequate at this time. However, NP and NPEs are likely early candidates for additional investigation when more sensitive methods for testing and assessment of endocrine-disrupting substances are developed.

Upon completion of such testing, evaluation of the potential endocrine-mediated adverse health effects of NP and NPEs should be considered a priority.

### **3.4.4 Conclusions on benefits of integration**

#### **Conclusions on benefits already present in EU-RAR**

None.

#### **Additional benefits of integration**

1. Integration strengthens the association of exposure-effects relationships in one species if the same exposure-effect relation is also observed in other species. For example, mechanisms of action in one fish species were confirmed with other fish species. Trout, salmon and killifish all respond to NP by making vitellogenin. Mechanisms of action in one rodent species were also confirmed with other rodent species (e.g., mice and rats responded similarly when exposed to NP).
2. Endpoints could be the same between species. However, data concerning an endpoint in one species are often not comparable or extensive enough (or not relevant enough) to make a useful comparison possible with the same endpoint of another species.
3. Mechanisms of action are confirmed by looking across the species barrier. It is even possible to integrate ecological data and human health data. Table 4 and Table 5 summarize the available *in vivo* and *in vitro* data. These tables support the findings that a) NP displaces estradiol and binds to the ER in both fish and mammals; and b) that NP influences reproductive organs in fish and mammals.

#### **Conclusions missed by not integrating**

The aim of an integrated risk assessment is to detect and identify adverse effects on humans and the ecosystem. There are enough data to set maximal safe concentrations of NP in water for fish and daphnia. For amphibians no estrogenic data are presented in either the EU and Environment Canada risk assessment reports. There is also enough experimental data from rats to set a maximal safe dose of NP for humans. There is sufficient information on reported adverse effects on mammals to be concerned about the potential estrogenic effects of NP on humans. This means that the risk assessments on the subject of estrogenic effects are fulfilling their purpose of identifying risk to men and ecosystems. As it is, there is a lot of information on dose-effect relations (on endocrine effects) for human health and for ecological assessments on NP. This reduces the need to look over species boundaries and between areas of expertise and specialization. It appears as if the separate risk assessments are quite complete on the subject of estrogenic effects for the purpose of establishing no-effect concentrations for fish and mammals. Limited data is available in the risk assessments on mechanisms of action of NP in various species.

Table 4 summarizes some of the *in vitro* and *in vivo* data on the ecological effects of NP. An attempt is made to combine ecological data with human health data to confirm known information or to find new insights. Cited references are listed in the EU-RAR.

		<b>Table 4: Ecological Test Data (Source: EU-RAR, 2002)</b>
<b>Ecological Test Data</b>	<i>In Vivo</i>	White <i>et al.</i> (1994) found that nonylphenol showed competitive displacement of estrogen from its receptor site in rainbow trout ( <i>Oncorhynchus mykiss</i> ).
		Concentration of 50 µg/L in water induces the expression of intersex-alterations in killifish.
		Jobling <i>et al.</i> (1996) found statistically significant reductions in testis size, expressed as gonadosomatic index (GSI). Histological examination of the testes showed that control fish had actively developed testes with a predominance of spermatocytes type A. The fish exposed to nonylphenol had a significantly higher proportion of spermatogonia type A than controls. A second experiment conducted, when the testes were more developed, examined a dose-response relationship for the two effects using nonylphenol. A significant stimulation of blood vitellogenin levels was seen after exposure to 20.3 µg/l but not at 5.02 µg/l, which was the NOEC for this effect. A significant reduction in GSI relative to controls was seen at 54.3 µg/l but not at 20.3 µg/l which was the NOEC for testicular growth.
		Concentration of 1 µg/L in water induces vitellogenin mRNA production in rainbow trout (Fent <i>et al.</i> , 1999).
		Elevated levels of blood vitellogenin in rainbow trout <i>in vivo</i> exposed to NP for 3 weeks, ranged 0.24-54.3 µg/L. The levels of blood vitellogenin were found to be significantly elevated at concentrations of 20.3 µg/L (1 µg vitellogenin /ml; a tenfold increase over controls) and 54.3 µg/L (100 µg vitellogenin /ml; a 1000-fold increase over controls) (Harries <i>et al.</i> , 1995).
		Concentration of 10 µg/L in water induces vitellogenin production in rainbow trout (Jobling <i>et al.</i> , 1996).

<b>Table 4: Ecological Test Data (Source: EU-RAR, 2002)</b>	
	<p>Arukwe <i>et al.</i> (1997) found that NP treatment caused an increase in the 6<math>\beta</math>-, 16<math>\alpha</math>- and 17<math>\alpha</math>-hydroxylase activities in salmon liver microsomes. There was an apparent dose-related decrease in the hydroxylase activities of liver microsomes. Reductions of activities were seen in the 7-ethoxyresorufin-O-deethylase (EROD) activity and the UDP-glucuronosyl-transferase activities.</p> <p>Immunochemical analysis of CYP1A, CYP2K-like and CYP3A-like proteins showed reductions in enzyme-linked immunosorbent assay absorbance levels. Plasma levels of estradiol-17<math>\beta</math> were found to be lowered.</p> <p>No relationships have been demonstrated between water quality characteristics (such as hardness and pH) and toxicity of NP.</p> <p>It was concluded that nonylphenol is capable of significantly perturbing components of androgen metabolism in daphnids at concentrations of <math>\leq 25 \mu\text{g/L}</math> (Baldwin <i>et al.</i>, 1997).</p> <p>Gray and Metcalfe (1997) found that a LOEC for incidence of testis-ova in the Japanese Medaka was 50 <math>\mu\text{g/L}</math>.</p> <p>Nimrod and Benson (1996) investigated the induction of serum vitellogenin in Channel Catfish. The mean serum vitellogenin levels were only significantly different (<math>p &lt; 0.05</math>) from controls in the high dose (237 mg nonylphenol/kg) fish. NP exposure was by intraperitoneal injection. After seven days, the serum vitellogenin level was determined.</p> <p>Christensen <i>et al.</i> (1995) dosed male flounders (<i>Platichthys flesus</i>) with nonylphenol by four intraperitoneal injections over a period of two weeks. Vitellogenin was detected in plasma of fish dosed with 10 mg/kg wet weight. Effects were also seen on plasma lipids (increase), protein (increase) and ninhydrin positive substances (decrease). Toxic effects (cell damage), as indicated by increased activity of the plasma enzyme GPT were also found.</p> <p>Ashfield <i>et al.</i> (1998) found that the ovosomatic index of female juvenile rainbow trout was found to be significantly (<math>p &lt; 0.05</math>) elevated in the 30 <math>\mu\text{g/L}</math> NP group.</p>
<i>In Vitro</i>	<p>White <i>et al.</i> (1994) reported that nonylphenol can stimulate vitellogenin secretion, <i>in vitro</i>, at concentrations of <math>10^{-6}</math> M (220 <math>\mu\text{g/L}</math>) and above in hepatocytes from rainbow trout.</p>

Table 5 summarizes some of the *in vitro* and *in vivo* data on the human health effects of NP. Cited references are listed in the EU-RAR.

		<b>Table 5: Human Health Tests Data (Source: EU-RAR, 2002)</b>
<b>Human Health Tests Data</b>	<i>In Vivo</i>	NP and NP2EO activated the estrogen receptor and had some estrogenic activity <i>in vitro</i> . NP was uterotrophic or induced other effects indicative of estrogenic activity in several studies <i>in vivo</i> . However, these compounds were between three and five orders of magnitude less active in this regard than estradiol. In addition, NP was estrogenic only at relatively high dose levels; for example, other effects (on renal histopathology) were observed at three times lower doses of NP than those in estrogen responsive tissues in the multigeneration study in rats (i.e., 12 vs. 50 mg/kg-bw).
		The influence of nonylphenol on growth and cell proliferation and of the mammary gland has been investigated in rats in two studies using non-standard methods. In the group receiving the highest dose of nonylphenol there was a 1.5-fold increase in the number of mammary structures and a fourfold increase in the number of cells/16 mm <sup>2</sup> area, compared with the vehicle control group (Colerangle and Roy, 1996).
		No developmental effects were reported at any dietary level; however, a range of effects on endocrine-regulated endpoints, including delayed vaginal opening, were observed at 650 and 2000 ppm. LOEL: 12–18 mg/kg-bw per day in males, 16–21 mg/kg-bw per day in non-lactating females or 27–30 mg/kg-bw per day in lactating females (NTP, 1997; Chapin <i>et al.</i> , 1999).
		NP is weakly estrogenic. NP increased uterine weight in immature or ovariectomized rats and in mice following oral administration of 50 mg/kg-bw per day and above and following subcutaneous and intraperitoneal administration (Lee and Lee, 1996; Shelby <i>et al.</i> , 1996; CMA, 1997; Coldham <i>et al.</i> , 1997; Laws and Carey, 1997; Odum <i>et al.</i> , 1997).
		Absorption from the gastrointestinal tract is initially rapid, and probably extensive. The major metabolic pathways are likely to involve glucuronide and sulphate conjugation, and there is evidence of extensive first pass metabolism of nonylphenol absorbed through the gastrointestinal tract. Because of first pass metabolism, the bioavailability of unconjugated nonylphenol is probably limited following oral exposure, at no more than 10-20% of the administered dose. Nonylphenol is distributed widely throughout the body, with the highest concentration in fat.
	<i>In Vitro</i>	In MCF-7 human breast cancer cells, cell proliferation was stimulated by NP at concentrations between 0.1 and 10 µM (22 and 2203 µg/L) (White <i>et al.</i> , 1994; Villalobos <i>et al.</i> , 1995; Blom <i>et al.</i> , 1998).
		In a proliferation test with MCF-7 and ZR-75 cells, from 10 µM/L in the medium the cells accelerated their growth.
		In <i>in vitro</i> studies, NP activated the estrogen receptor with a potency 5000–7000 times less than that of 17β-estradiol (Routledge and Sumpter, 1996; Gaido <i>et al.</i> , 1997; Odum <i>et al.</i> , 1997).

### 3.5 Risk Assessment for wildlife

#### 3.5.1 Introduction

There is a lack of measured exposure data and data on NOECs for wildlife. Both exposure and NOECs are needed to be able to quantify the risks for wildlife.

##### 3.5.1.1 Measuring NOECs for wildlife

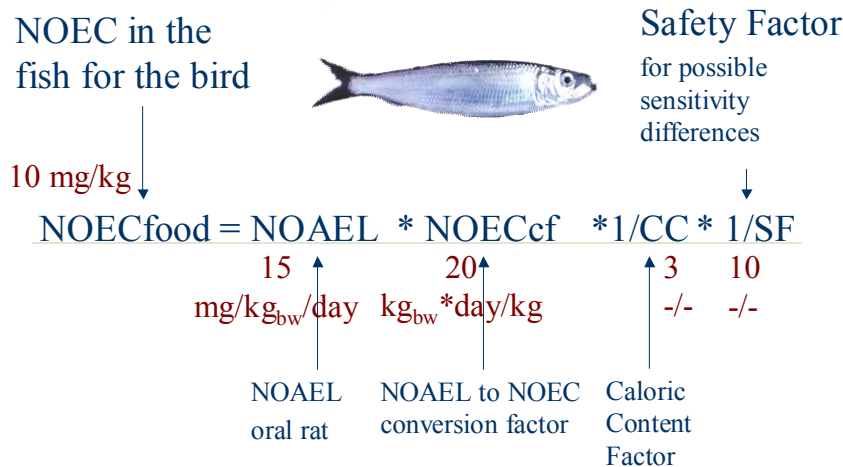
Direct measures of NOECs for most wildlife species are unavailable. NOECs are difficult to derive indirectly via measurements of concentrations in cadavers or unhatched eggs of birds of prey. Establishing a relation between concentrations in prey and predator population size and health is unlikely to succeed because cause and effect are difficult to prove.

##### 3.5.1.2 Extrapolating rat NOAELs to NOAEL for humans

A rat LOAEL of 15 mg/kg/day (repeated dose for 20 weeks) based on histo-pathological changes in the kidneys and a rat NOAEL of 15 mg/kg/day based on minor perturbations in the reproductive system of offspring have been reported. In the case of NP, it is assumed that man and rat are equally sensitive and direct extrapolation from rat to human is allowable, reaching a N(L)OAEL of 15 mg/kg/day for oral exposure. In the risk characterization the Margin Of Safety will be used, taking into account uncertainties due to inter- and intraspecies differences as well as differences in dosing schemes compared to real-life situations.

##### 3.5.1.3 Extrapolating rat NOAEL to NOECs for wildlife

A lot of data are available from rodent lab testing. Based on the NOAEL<sub>oral</sub> of the rat using NOECs<sub>oral</sub> for predators, oral exposure can be calculated as shown in Figure 5.



**Figure 5:** The NOAEL<sub>oral</sub> of the rat is based on reproductive effects. A safety factor of 10 is applied in case birds are intrinsically more sensitive to nonylphenol than rats. It is assumed that the feed of laboratory animals is three times more caloric than the feed eaten by wild animals. The calculated concentration of 10 mg/kg in prey fish is the expected concentration that will cause no detrimental effects in the bird of prey on the long term.

Due to the uncertainties in extrapolation factors, safety factors are necessary, and therefore, low avian and mammalian NOECs are derived. Additional safety Factors could also be applied for differences (e.g. hibernation, migration, or other periods of higher or lower metabolic activity) in caloric content of the food between species, and in route of exposure.

### **3.5.2 Potential benefits by integration ERA and HHRA knowledge**

1. Derivation of NOECs for untested organisms from mammalian toxicity data.
2. Estimates of exposure in wildlife species.

### **3.5.3 Risk Assessment Data (Source: EU-RAR, 2002)**

#### ***3.5.3.1 Environmental Data***

Only toxicity studies reporting on dietary and oral exposure are relevant. Secondary poisoning effects on bird and mammal populations rarely become manifested in short-term studies. Therefore, results from long-term studies are strongly preferred (such as NOECs for mortality, reproduction or growth). But, if no adequate toxicity data for mammals or birds are available, an assessment of secondary poisoning cannot be made. Nonylphenol has been shown to bioconcentrate in aquatic species. No toxicity data are available on avian species; thus a PNEC is derived from laboratory mammal data. A NOAEL of 15 mg/kg body weight was found for reproductive effects. Using appropriate conversion factors to allow for the fact that the caloric content of a laboratory diet is higher than that of the diet of fish-eating mammals and birds, this NOAEL is equivalent to a daily dose of 100 mg/kg food. Using an additional safety factor for reproductive effects, PNEC<sub>oral</sub> has been calculated as 10 mg/kg food.

#### ***3.5.3.2 Human Health Data***

The calculations of human intake from air, water and food assume absorptions of 75% by inhalation and 100% from the oral route. Exposure via the air makes little contribution to the overall dose. The oral uptake may be an overestimate but the amount taken up is compared directly with the rat oral LOAEL for repeat dose-effects and NOAEL for reproductive toxicity affect (both of 15 mg/kg/day) which represents the dose given rather than the amount taken up. Based on these estimates, an human NOEL of 15 mg/kg/bw have been calculated.

### **3.5.4 Conclusions on benefits of integration**

#### **Conclusions on benefits already present in EU-RAR**

1. NOECs for predators (although uncertain) are derived using human-toxicological data.
2. Uncertainty in the extrapolation from species to species is acknowledged.
3. Approaches using safety assessment factors are possible.
4. Exposure of fish-eating birds/mammals and earthworm-eating birds/mammals can be estimated or determined through monitoring based on the model as used in the EU-RAR conceptual.

### **Additional benefits of integration**

None can be derived for the data available.

### **Differences between conclusions of the EU and Canada**

- The Canadian-EPA does not consider secondary poisoning an issue, due to the low bio-accumulative potential of NP. The EU-RAR, however, mentions the risk of secondary poisoning at local sites.

### **Opportunities missed in the EU-RAR**

- Higher trophic levels of fish-eating fish are not considered.
- The current guideline for extrapolation is focused on earthworm-eating birds and fish-eating birds. Extrapolation should also include small wild predators such as badgers, foxes, ferrets, owls and hawks and terrestrial prey such as mice, rabbits and squirrels. Larger relevant organisms such as deer and wild ranging animals should also be included.

## 4. CONCLUSIONS

### 4.1 Advantages of Integration

The advantages of integration for nonylphenol are summarized per area of integration in Table 6. The partly integrated EU nonylphenol risk assessment was taken as the basis for this integrated risk assessment. Some benefits of integration might have been stronger if one separate ecological and one separate human health risk assessment had been available and merged into one integrated risk assessment. The problem formulation and the execution of the risk assessment were already standardized in the EU approach. This made it difficult to specific benefits of integration in these phases.

<b>Table 6: Benefits of Integration for Nonylphenol (Per Area of Integration)</b>	
<b>EXPECTED BENEFITS</b>	<b>IDENTIFIED BENEFITS</b>
This column contains the expected benefits of risk assessment per phase of the assessment.	This column contains the identified benefits of risk assessment. Empty cells mean that the expected benefits were not found.
<b>Problem formulation</b>	
<u>Assessment questions</u>	
<ul style="list-style-type: none"> <li>• Issues that are critical for both humans and the environment are more easily identified. A deeper, daily, working integration would greatly increase the chance of serendipitous recognition of problems for which evidence in any one sector is limited, but when all data are considered together, cause for concern may become evident.</li> <li>• Risks to humans are adequately considered through evaluation of risks to other organisms that influence human health and welfare.</li> <li>• Consistency in the spatial and temporal scope, e.g. with regard to the information and processes used, is established.</li> <li>• Data and knowledge gaps are identified at an early stage.</li> </ul>	Without integrating, the ecological and human health risk assessments may have differed with regard to spatial and temporal scales considered, the degree of conservatism, the terminology used, the exposure scenarios for environmental exposure and the default values used. In addition the evaluation of the quality of the database may have differed.

<b>Table 6: Benefits of Integration for Nonylphenol (Per Area of Integration)</b>	
<u>Impetus for the assessment</u>	Not relevant, since the stressor, nonylphenol, had been specified.
<u>Assessment endpoints</u>	
<ul style="list-style-type: none"> <li>• Susceptible endpoints in animals or ecosystems could indicate unidentified endpoints in humans</li> </ul>	
<ul style="list-style-type: none"> <li>• Knowledge on the fate of the compound and the target organisms or organs(s) can predict potential effects in ecosystems or organs, respectively.</li> </ul>	
<ul style="list-style-type: none"> <li>• Ecologists and human health assessors can discuss the relevance of all chosen endpoints.</li> </ul>	
<u>Conceptual model</u>	
<ul style="list-style-type: none"> <li>• Both human and ecological risk assessors scrutinize the quality of the common pathways to humans and ecology from the sources of the stressor.</li> </ul>	
<ul style="list-style-type: none"> <li>• Consensus between ecologists and human health assessors on the fate of the stressor is established.</li> </ul>	
<ul style="list-style-type: none"> <li>• Indirect exposure pathways via the environment to the humans are more likely to be identified, as humans are modeled as just one more receiving species in the web of exposure pathways.</li> </ul>	
<ul style="list-style-type: none"> <li>• When relevant and possible, multiple sources of exposure such as consumer products, emissions, and natural emissions are incorporated.</li> </ul>	
<ul style="list-style-type: none"> <li>• Only one conceptual model is produced; the ecological conceptual model and the human health conceptual model are one and based on the same assumptions and data.</li> </ul>	
<u>Analysis plan</u>	
<ul style="list-style-type: none"> <li>• Enhanced efficiency and reduced costs as only data for one transport and fate model are needed. Emission data, physico-chemical properties, fate and degradation data are only needed once.</li> </ul>	Emission data, physico-chemical properties, fate and degradation data are searched for, evaluated and used only one time. Measured data are also searched for and evaluated once.

<b>Table 6: Benefits of Integration for Nonylphenol (Per Area of Integration)</b>	
Ecological and human exposure models use the same input, that is, the results of one transport and fate model. By integrating, decisions are based on more data and the available data are used more extensively.	
<ul style="list-style-type: none"> <li>It helps identify targeted research needs (e.g., where do ecological and human health risk assessors lack information).</li> </ul>	This benefit is not verified but very likely.
<ul style="list-style-type: none"> <li>Ecological results and human health results adhere to the same quality restrictions.</li> </ul>	The quality of the IRA is ensured with one analysis plan. Conclusions for ecological or human health are equally strong and equally reliable: questioning the quality of the ecological conclusions means questioning the quality of the human health conclusions. One cannot selectively use data in the integrated assessment.
<ul style="list-style-type: none"> <li>Ecological results and human health results are presented using the same terminology and if possible use the same method to express the risk and the uncertainty. This facilitates comparison between the ecological results and the human health results.</li> </ul>	Use of the same risk representations (e.g., MOS) facilitates comparison between the ecological results and the human health results.
<ul style="list-style-type: none"> <li>If the assessment will be iterated, an integrated approach might foster the development of parallel ties, which use common data and models.</li> </ul>	
<b>Emission Sources, Environmental Concentrations, and Exposure Estimates</b>	
<ul style="list-style-type: none"> <li>Routes of emission, distribution and exposure pathways are translated into model equations describing how the stressor reaches the organisms via diverse routes of exposure. Human and ecological risk assessors can discuss which models and which input values are appropriate and choose a common approach.</li> </ul>	A common modeling approach is applied to the exposure estimation of nonylphenol for ecological receptors and humans leading to a coherent assessment and avoiding duplication of work. For instance, the same estimated emission and background levels are used in the exposure estimation for environmental organisms and for human beings. The estimation of exposure of man via fish is estimated from the surface water concentration. The same concentrations also determine the risks for

<b>Table 6: Benefits of Integration for Nonylphenol (Per Area of Integration)</b>	
	aquatic organisms.
<ul style="list-style-type: none"> <li>The type of model can be of the appropriate space- and time scales with regard to both the human and the environmental risk assessment.</li> </ul>	An integrated exposure model is used.
<ul style="list-style-type: none"> <li>Monitoring data for emissions, environmental compartments, biota, food, feed and drinking water can be shared.</li> </ul>	The available monitoring data are shared. This avoids the use of other data of different quality in independent assessments. It results in a common approach towards the analysis of the accuracy, specificity and relevance of these data.
<ul style="list-style-type: none"> <li>The whole life cycle of the stressor and all possible sources of emission will be considered.</li> </ul>	Emissions to the environment from all potential sources are considered.
<ul style="list-style-type: none"> <li>Local sites with high concentrations of a chemical can cause potential adverse ecological effects with indirect effects on humans. These effects are not exemplified in an ERA. This means that such effects of (e.g., a massive fish extinction) are not estimated. Integration can solve this problem.</li> </ul>	The EU approach predicts concentrations on different spatial scales. This helps to identify potential local high concentrations in the environment of man and animals living near a source of NP. The EU approach helps to identify problems due to high background exposure on a continental scale. By integrating several spatial scales, more information is generated than by only using one scale of space.
<ul style="list-style-type: none"> <li>Coherent conclusions can be drawn with regard to additional analytical activities necessary for higher tier human and ecological risk assessment.</li> </ul>	This promotes the best use of the available resources and an integrated sampling strategy.
<ul style="list-style-type: none"> <li>A common approach towards uncertainty analysis can be developed.</li> </ul>	The exposures of humans or the environment are uncertain due to emission uncertainties. In this case, integration does not help against a lack of data.
<ul style="list-style-type: none"> <li>A common approach to the evaluation of data quality, completeness, and relevance is developed.</li> </ul>	In the case of the EU approach, guidelines on when to accept and when to decline data have been formulated.
<b>Toxicokinetics</b>	
<ul style="list-style-type: none"> <li>ADME (Absorption, Distribution, Metabolism, and Excretion) data and toxicokinetic models for laboratory animals and man may be extrapolated to wildlife and vice versa.</li> </ul>	Based on the available information for nonylphenol, the combination of data from the ERA and HHRA does not result in additional insights or remarkable benefits on the topic of toxicokinetics.

<b>Table 6: Benefits of Integration for Nonylphenol (Per Area of Integration)</b>	
<ul style="list-style-type: none"> <li>• Development and improvement of Physiologically Based Pharmacokinetic (PB/PK) modeling by combining data sets of different species could increase interspecies extrapolation.</li> </ul>	
<ul style="list-style-type: none"> <li>• Identification of metabolic pathways observed in environmental biota (ERA) and in laboratory mammals (HHRA) may strengthen each other.</li> </ul>	
<ul style="list-style-type: none"> <li>• Information about elimination rates in environmental biota or in mammalian test organisms may point to the expectation that a chemical may have a short or long half-life in organisms.</li> </ul>	
<ul style="list-style-type: none"> <li>• Information about accumulation in specific biota may point to relevant uptake routes for humans. For example: if a particular chemical hardly accumulates in a certain food product there will be no need to take this route of exposure into account in the human health risk assessment. In the opposite case, chemical accumulation might occur in an unexpected manner. Such information could be used both to find relevant routes of exposure for humans and to find biota that is likely to be more at risk.</li> </ul>	
<ul style="list-style-type: none"> <li>• Biomarkers developed for one species may be useful for exposure/effect assessment in other species. Biomarkers such as DNA-adduct formation or the occurrence of certain metabolites in urine could be used to demonstrate the presence of the stressor and quantify exposure.</li> </ul>	

<b>Table 6: Benefits of Integration for Nonylphenol (Per Area of Integration)</b>	
<b>Estrogenic Effects</b>	
<u>Understanding of mechanism of action and endpoints</u>	
<ul style="list-style-type: none"> <li>Mechanisms of action can be confirmed by looking across the species barrier. Estrogen/steroid modes of action in fishes and mammals can be the same, as estrogen receptors are often highly conserved in structure.</li> </ul>	<p>Mechanisms of action are confirmed by looking across the species barrier. It is even possible to integrate ecological data and human health data.</p> <ul style="list-style-type: none"> <li>NP displaces estradiol and binds to the ER in both fish and mammals.</li> <li>NP influences reproductive organs in fish and mammals.</li> </ul>
<ul style="list-style-type: none"> <li>Detection of critical pathways from target site to endpoint across species may be possible.</li> </ul>	
<ul style="list-style-type: none"> <li>Knowledge on links between molecular events and endpoints is improved.</li> </ul>	<p>Endpoints could be the same between species; but data concerning an endpoint in one species are often not comparable, relevant, or extensive enough to make a useful comparison with the same endpoint of another species.</p>
<ul style="list-style-type: none"> <li>Effect found in wildlife populations might identify new endpoints. This could assist in identifying emerging new risks for humans. This also reduces the changes of overlooking critical effects.</li> </ul>	
<u>Dose-effect relationships</u>	
<ul style="list-style-type: none"> <li>Integration strengthens associations of exposure-effects in one species if the same exposure-effect relation is also observed in other species.</li> </ul>	
<u>Extrapolation from human health data to wildlife data</u>	
<ul style="list-style-type: none"> <li>Improved cross-species extrapolation might reduce or validate extrapolation factor values.</li> </ul>	
<ul style="list-style-type: none"> <li>In the absence of toxicity data for predators in the ecological risk assessment, the toxicity data for rodents (generated in the framework of the human health risk assessment) can be used to evaluate the hazard to predators.</li> </ul>	<p>See Risk Assessment of Wildlife below.</p>

<b>Table 6: Benefits of Integration for Nonylphenol (Per Area of Integration)</b>	
<ul style="list-style-type: none"> <li>• Effects found in wildlife populations might warn for low dose-effect in human populations. One sensitive species may serve as “early warnings” for other organisms, including humans.</li> </ul>	Comparing human and ecological dose-effect data has identified no new endpoints.
<b>Risk Assessment of Wildlife</b>	
<ul style="list-style-type: none"> <li>• NOECs for untested organisms, such as wildlife from mammalian toxicity data, are derived.</li> </ul>	<ul style="list-style-type: none"> <li>• NOECs for predators, although uncertain, are derived using exposure data from human health assessments.</li> <li>• Extrapolation problems from species to species are acknowledged but not solved.</li> <li>• Secondary poisoning in fish-eating birds and earthworm-eating birds would be detected or predicted.</li> </ul>
<ul style="list-style-type: none"> <li>• The exposure of wildlife can be calculated.</li> </ul>	

#### **4.2 Evaluation of the Scientific Benefits and Drawbacks of this Nonylphenol IRA**

The expected benefits of integration would involve enhanced coherent expression of assessment results, enhanced interdependence of the results, identification of sentinel organisms, enhanced scientific quality of the assessment results and an increased efficiency in using and generating data (HERA, 2003).

##### **Coherent expression of assessment results**

Human and ecological results are expressed in risk characterizations ratios: PEC/PNECs for environmental species and margins of safeties (MOS) for humans. Throughout the assessment the same type of risk characterization terms, such as PNEC, are used. The final conclusions of the risk assessment reports in the EU (concern and risk reduction measures needed, no concern, more data required) are based on decision rules with these risk characterization ratios as points of departures. Such a coherent expression is easier to communicate.

##### **Interdependence of the results**

The conceptual model in the EU risk assessment is already partly integrated. This approach means that where overlaps in exposure routes and the calculation and measurements of environmental concentrations occur, human and ecological exposures are calculated with the same tools and data. Results are harmonized with regard to concentrations, contact media and routes of exposures. With regard to the estrogenic effects of nonylphenol, the mechanism of action is confirmed by looking across species barriers and integrating the ecological data with

human data. Additionally, effects data on mammals are shared for the determination of no-effect levels on humans and in wildlife.

### **Identification of sentinel organisms**

Although no new sentinel organisms were identified, the integrated risk assessment does lead to an enhanced understanding of the impact of nonylphenol as an endocrine disruptor on different species, including humans. When bioaccumulation takes place, NP concentrations become higher in the predatory fish (resulting in increased vitellogenin production in male fish). Elevation of vitellogenin levels can thus possibly serve a sentinel process.

### **Enhanced scientific quality of the assessment result**

- Concentrations in humans and organisms in the environment are harmonized by using the same measured or modeled concentrations in the environmental compartments. Human and environmental exposure pathways are combined in one conceptual model. Human and top predator exposures are integrated via calculation of the concentrations of NP products in the same species. The EU approach integrates direct and indirect exposure. Humans and ecosystem are both included in the model, with common sources and the same fate and transport model.
- Integration does not increase the quality of the dose-effect characterization in the risk assessment for humans. The environmental risk assessment can gain from the human health risk assessment by extrapolations from rat data to other mammals.
- Integrating in the area of toxicokinetics is unlikely to yield benefits given the minimal amount of available data. Integration via internal dose modeling, for example, is not feasible due to a lack of data. The only additional insight gained here is that the metabolism of nonylphenol proceeds through similar pathways across species, including humans. Overall the data are rather limited for integration.
- Only the most general overlap in mechanism of action of nonylphenol has been found, using data from the available risk assessments.

In summary, because no new data were used and the EU-RAR is already partly integrated, the conclusions of the EU-RAR have not changed in this report. The benefits of integration apparent in the EU-RAR have been highlighted. The ecological and human assessment results are made interdependent and use the same data as much as possible.

### **Efficiency in using and generating data**

The integrated approach means that where overlaps in exposure routes, and in calculation and measurements of environmental concentrations occur, human and ecological exposure is calculated with the same tools and data. Available measured concentrations and emission data are used for both the human and ecological exposure assessments. Data searches and the evaluation of the quality of these data occur only once. Data requirements for the ecological and human risk assessments could be further harmonized (e.g., with regard to further elucidation of the mechanism of action, dose-effect relationships and inter- and intraspecies variation of the estrogenic effect).

## **Drawbacks**

No drawbacks were identified. Integration does increase the need for ecologists and human health assessors to meet and spend time in discussion, to generate a common vision. Based on these decisions, risk assessments can be made more efficient and complete.

### **4.3 Costs and Benefits in Economics Terms**

It was impossible to quantitatively estimate the differences in costs and benefits in economic terms between independent ERA and HHRA assessments of nonylphenol and the integrated risk assessment in this exercise. Therefore a more qualitative approach was followed. The IPCS framework for integrated risk assessment (see Table 7) can be used as a checklist of the steps to conduct a solid integrated risk assessment. Almost all the steps taken to make an ERA are also done when making a HHRA. The assumption is made that the more steps the ERA and HHRA have in common, the greater the reduction in costs will be in making an IRA. The benefits gained and costs saved are thus proportional to the measure of similarity per phase or step. This measure of similarity between ERA and HHRA is quantitatively expressed using ratings from 0 to 3 (taking the assessment of nonylphenol as example). Zero means no similarity; 1 means some similarity in terminology and types of decisions to be taken; 2 means multiple commonalities in terminology, types of decisions to be taken, and data requirements and type of experts and knowledge needed; and, 3 stands for a high degree of similarity.

From Table 7 it can be concluded that a high gain in benefits and reduction of costs can be expected on approximately 50% of the steps taken. Nevertheless, some caution is needed because:

1. This is a limited analysis.
2. If animal testing and data collection on environmental concentrations consume the bulk of the budget then a relatively high reduction in the budget for the other steps is only a small reduction in absolute numbers on the total budget.
3. Before any testing and measurements are done, data searches are done to find available data. If a HHRA is done before an ERA, then the available data from that risk assessment will be used in the ERA. This reduces the costs of the ERA and is a sensible practice.

**Table 7<sup>†</sup>: Steps or Data Needed per Phase***Based on the IPCS-Framework (HERA 2003)*

<b>Problem formulation</b>	<b>Ecological</b>	<b>Human</b>	<b>IRA</b>	<b>Similarity</b>
planning dialog	X	X	X	2
management goals	X	X	X	2
purpose and scope	X	X	X	2
available resources	X	X	X	?
identification of the effects caused by the stressor	X	X	X	3
preliminary identification of endpoints	X	X	X	3
<b>Characterization of exposure</b>				
data completeness	X	X	X	3
stressor characteristics	X	X	X	3
sources and emissions	X	X	X	3
industrial use	X	X	X	3
consumer usages	O	X	X	0
distribution pathways	X	X	X	3
quantitative / computer model	X	X	X	3
environmental transport and fate, degradation	X	X	X	3
external exposure model	X	X	X	2
internal exposure model	?	?	?	?
phrasing of uncertainties	X	X	X	3
choice of hyper-conservative or realistic scenario	X	X	X	3
<b>Characterization of effects</b>				
identification of mode of action	X	X	X	3
exposure-response analysis:				
plants	X	X <sup>#</sup>	X	3
fish	X	X <sup>#</sup>	X	3
mammals	X	X <sup>#</sup>	X	3
birds	X	X <sup>#</sup>	X	3
humans via rats	O	X	X	0
evaluation of data	X	X	X	3
evaluation of time scales of effect	X	X	X	2
extrapolation factors	X	X	X	2

<sup>†</sup> This table is meant to be completed in a general manner. X means this step is needed in the RA. O means this step is not needed in the RA. ? means this step could be useful if enough data are present. # means food and resource insurance for humans. See text for explanation of numbers.

<b>Table 7<sup>†</sup>: Steps or Data Needed per Phase</b>				
<i>Based on the IPCS-Framework (HERA 2003)</i>				
<b>Risk characterization</b>				
combine exposure and dose-effect data	X	X	X	3
uncertainty estimation	X	X	X	3
‘translate’ results and conclusions for risk managers and stakeholders	X	X	X	3
<b>Risk management</b>				
selection of feasible management measurements	X	X	X	1
consideration of consequences of management measurements	X	X	X	1
<b>Stakeholder participation</b>				
Stakeholders discuss endpoints and review assessment results.	X	X	X	2
<b>Risk communication</b>				
explanation of legal, policy, time and resource constrains to stakeholders	X	X	X	3
expression of type of risks and explanation of uncertainties to stakeholders	X	X	X	3

#### 4.4 Evaluation of the IPCS Framework

Since the EU ecological and human risk assessments of nonylphenol are already partly integrated and fully reviewed by EU-experts, the IPCS Framework could not be tested to its full extent. An important element of the framework (i.e., the interaction between ecological and human risk assessors with risk managers and stakeholders) was not investigated.

The Framework provided guidance since it helped to generate a list of steps needed to perform an IRA and can be used as a starting point to derive areas of integration. For example, the problem formulation phase is well-explained and divided into smaller well ordered steps, such as assessment questions, impetus for the assessment and assessment endpoints. Each step of the Framework was checked in this report for the possibility to improve the EU assessment. Overall, the Framework appears to give a complete discussion of all essential elements. The Framework is written as a general guidance. It would benefit from giving more detailed guidance on several issues (such as calculations, assumptions, decisions, and data selection per topic and per stressor). A list of references and examples illustrating the various steps in an integrated risk assessment would be useful. Overall, the Framework provides good guidance for the harmonization of risk assessments worldwide.

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## 6. APPENDICES

### 6.1 Appendix 1: *In vivo* data on ecological endocrine effects (Source: EU-RAR, 2002)

Individual references are listed in the EU-RAR.

Jobling *et al.* (1996) exposed two-year-old male rainbow trout (*Oncorhynchus mykiss*) to nonylphenol at 30 µg/l (nominal concentration) in a flow through system for three weeks. Measured concentrations of nonylphenol were  $36.81 \pm 2.4$  µg/l throughout the experiment. Exposure was conducted in May, when growth of the testes was at an early stage. Blood samples were taken at the beginning and end of the exposure period. After three weeks, the fish were killed and testicular weight measured. Nonylphenol was found to stimulate production of vitellogenin, by a factor of 100 to 1000 times compared to controls. Statistically significant reductions in testis size, expressed as gonadosomatic index (GSI) were also noted. Histological examination of the testes showed that control fish had actively developed testes with a predominance of spermatocytes type A. The fish exposed to nonylphenol had a significantly higher proportion of spermatogonia type A than controls. A second experiment conducted when the testes were more developed, examined the dose-response for the two effects. A significant stimulation of blood vitellogenin levels was seen after exposure to 20.3 µg/l but not at 5.02 µg/l, which was the NOEC for this effect. A significant reduction in GSI relative to controls was seen at 54.3 µg/l but not at 20.3 µg/l which was the NOEC for testicular growth.

Baldwin *et al.* (1997) investigated the effects of exposure of *Daphnia magna* to nonylphenol in a three-week assay designed to look at the effects on the metabolism of the steroid hormone testosterone and any resulting effects on reproduction. Both acute and long-term exposures were used in the test. In acute tests, adult (ten-day-old) female daphnids were exposed to nonylphenol (25, 50 or 100 µg/L) for 48 hours. After the acute exposure, the daphnids were exposed to [<sup>14</sup>C] testosterone for a further sixteen hours and analysed for total radioactivity. The presence of radiolabelled metabolites in the water was also determined. Effects of exposure to nonylphenol on reproduction were investigated in a three-week static renewal toxicity test. Again, after the three-week exposure, the daphnids were exposed to [<sup>14</sup>C] testosterone for a further sixteen hours to investigate the effects on steroid hormone metabolism. After 48 hours of exposure to nonylphenol at 100 µg/L, a significant increase (p=0.01) over the controls was seen in the accumulation of [<sup>14</sup>C] testosterone and/or its metabolites in the daphnids. No significant effect was seen at nonylphenol concentrations of 25 or 50 µg/L. More detailed investigation of the metabolic elimination products indicated that the increased accumulation of androgens in the daphnids was a result of a decrease in the production of the major testosterone elimination product (testosterone-glucose) and an increase in the production of reduced/hydrogenated metabolites that are preferentially retained in the daphnids. These effects were seen at all exposure concentrations and were concentration-related (although the effects were not always statistically significant at nonylphenol concentrations of 25 µg/L). It was concluded that nonylphenol is capable of significantly perturbing components of androgen metabolism in daphnids at concentrations of  $\leq 25$  µg/L. In the three-week reproduction assay, nonylphenol concentrations of up to 100 µg/L had no effect on survival of parental daphnids. The number of off-spring produced was reduced on exposure to 50 or 100 µg/L, but this reduction was only statistically significant (p=0.05) at 100 µg/L. The reproductive chronic value derived from these data was 71 µg/L (geometric mean of the NOEC and LOEC for reproduction) and this

concentration was estimated to reduce the elimination of testosterone by approximately 50%. The results indicate that nonylphenol can cause effects on steroid hormone metabolism that may contribute to its reproductive toxicity (Baldwin *et al.*, 1997).

The effects of nonylphenol exposure on both the asexual and sexual reproduction of *Daphnia galeata mendotae* has been studied over 30 days of exposure (Shurin and Dodson, 1997). Four parameters (averaged over a female's lifetime) were examined: a) number of female offspring; b) number of male offspring; c) number of ephippia and d) number of developmentally abnormal male and female offspring. The laboratory conditions used induced the production of all three types of offspring (males, females and ephippia) and the exposure media were renewed every 48 hours. The nonylphenol concentrations used were 10, 50 and 100 µg/L. The results from the test were complicated due to the fact that different responses were seen in the solvent control (acetone at 80 µg/L) and the medium control. The daily production of female offspring/adult was found to increase over that seen in the medium control at the high concentrations (50 and 100 µg/L) of nonylphenol, but a similar increase was seen in the solvent control. No effects were seen on the daily production of male offspring/adult at any concentration and a slight decrease in the number of ephippia/adult was seen at high doses of nonylphenol. This latter effect was thought to be a result of increased adult mortality at the high nonylphenol concentrations. The daily production of deformed live offspring/adult was found to be related to nonylphenol exposure as a clear dose-response curve was seen and no such deformed offspring were seen in the two controls. The deformed offspring were of similar size to normal offspring but had forward curled tail spines and lacked, or had severely reduced, terminal setae on their second antennae, which reduced the swimming ability of the organism. This deformity was seen in 11% of live young at a nonylphenol concentration of 10 µg/L, and only animals that were prenatally exposed to nonylphenol exhibited this deformity.

Gray and Metcalfe (1997) investigated the sexual development of male and female Japanese Medaka (*Oryzias latipes*) exposed to nonylphenol from hatching to three months of age. The test used was a static renewal system (renewal every 72 hours for first month and then every 48 hours), using 30 fish per exposure concentration. Exposure was initiated one or two days post hatch. The nominal concentrations used were 10, 50 and 100 µg/L, but analysis indicated that these concentrations fell over the 48-hour or 72-hour renewal period and the mean measured concentration over the renewal period was around 55% of the nominal for 72-hour renewal and 66% for 48-hour renewal. Between 18 and 20 of the original 30 fish in each treatment and control survived to the end of the three-month exposure period. A statistically significant increase in mean body weight and length was found for the fish in the 10 and 50 µg/L groups when compared with controls. This was not apparent in the 100 µg/L treatment group. Histological examination indicated that males in the 50 and 100 µg/L had developed testis-ova, characterized by the presence of both testicular and ovarian tissue in the gonad. The incidence of this was six out of twelve males (50%) in the 50 µg/L treatment and six out of seven males (86%) in the 100 µg/L treatment. No incidence of testis-ova was found in the control group (twelve males) or the 10 µg/L treatment (ten males). The LOEC for this effect was therefore 50 µg/L. At 100 µg/L the authors suggested that sex reversal (male to female) may also be occurring as the ratio of males to females was different to that seen in controls or the 10 and 50 µg/L treatments. However this could also be due to different mortality patterns in the various treatments (i.e. greater mortality in male fish at 100 µg/L). It was also noted that the Japanese

Medaka have a relatively unique process of gonadal differentiation and development and it is not clear how these results relate to possible effects in other fish species.

Nimrod and Benson (1996) investigated the induction of serum vitellogenin in Channel catfish (*ictalurus punctatus*) by  $17\beta$ -estradiol, several synthetic estrogens and several suspected xenestrogens including nonylphenol. Juvenile fish (65-95 g) were exposed to each substance by intraperitoneal injection. After seven days, the serum vitellogenin level was determined. Fish exposed to nonylphenol at doses of 79 mg/kg and 237 mg/kg showed elevated serum vitellogenin levels when compared to controls. The response of individual fish was found to be very variable and the mean serum vitellogenin levels found in control, low dose and high dose groups were  $0.3\pm 0.4$  mg/ml,  $3.6\pm 3.4$  mg/ml and  $9.5\pm 5.7$  mg/ml respectively, however, this was only significantly different ( $p < 0.05$ ) from controls in the high dose (237 mg nonylphenol/kg) fish. The response from nonylphenol was much lower than that found with  $17\beta$ -estradiol by a factor of around 5000 (i.e. a 500 times higher dose of nonylphenol resulted in a ten times lower serum vitellogenin level compared with that seen with  $17\beta$ -estradiol).

Christensen *et al.* (1995) dosed male flounders (*Platichthys flesus*) with nonylphenol by four intraperitoneal injections over a period of two weeks. Vitellogenin was detected in plasma of fish dosed with 10 mg/kg wet weight. Effects were also seen on plasma lipids (increase), protein (increase) and ninhydrin positive substances (decrease). Toxic effects (cell damage), as indicated by increased activity of the plasma enzyme GPT, was also found.

Elevated levels of blood vitellogenin have been found in rainbow trout (*oncorhynchus mykiss*) exposed *in vivo* to nonylphenol for three weeks. The nonylphenol concentrations used were in the range 0.24-54.3  $\mu\text{g/L}$ . The levels of blood vitellogenin were found to be significantly elevated at concentrations of 20.3  $\mu\text{g/L}$  (1  $\mu\text{g}$  vitellogenin /ml; a tenfold increase over controls) and 54.3  $\mu\text{g/L}$  (100  $\mu\text{g}$  vitellogenin /ml; a 1000-fold increase over controls) (Harries *et al.*, 1995).

The effects of nonylphenol on steroid metabolizing enzymes from the liver have been studied using Atlantic Salmon (*Salmo salar*) (Arukwe *et al.*, 1997). Groups of six fish (approximately one year old and between 75 and 120 g in weight) were injected intraperitoneally with either 1, 5, 25 or 125 mg/kg bodyweight of nonylphenol (consisting of 85% para-isomers, and around 8-13% phenol, 1% tripropylene and 1% dinonylphenol) and then maintained at 10°C and 34‰ salinity for two weeks. Similar groups of fish were dosed with 5 mg/kg body weight of estradiol- $17\beta$  as positive control and the carrier solvent (vehicle control group). After the two-week period, various assays were carried out using liver microsomes collected from the exposed and control fish. The nonylphenol treatments caused an increase in the  $6\beta$ -,  $16\alpha$ - and  $17\alpha$ -hydroxylase activities in liver microsomes from the 1 mg/kg body weight groups (the increase was only statistically significant ( $p < 0.05$ ) compared with vehicle controls for the  $6\beta$ -activity). With increasing dose of nonylphenol, there was an apparent dose-related decrease in the hydroxylase activities of liver microsomes compared to vehicle controls. This decrease was statistically significant for  $6\beta$ -hydroxylase in the 25 mg nonylphenol/kg body weight group and for all activities in the 125 mg nonylphenol/kg body weight group. Reductions compared with vehicle controls were also seen in the 7-ethoxyresorufin-O-deethylase (EROD) activity (23-70% reductions were seen but they were only statistically significant in the 125 mg nonylphenol/kg

body weight groups) and the UDP-glucuronosyltransferase activities (decrease was not statistically significant). Immunochemical analysis of CYP1A, CYP2K-like and CYP3A-like proteins showed statistically significant 18%, 47% and 30% reductions in enzyme-linked immunosorbent assay absorbance levels respectively compared with vehicle controls in the 125 mg nonylphenol/kg body weight group. Plasma levels of estradiol-17 $\beta$  were found to be lowered by 24-43% compared with vehicle controls, but this decrease was only statistically significant in the 1 and 5 mg nonylphenol/kg body weight treated groups. The report concluded that nonylphenol may increase the activity of steroid-metabolising enzymes at low concentrations but decrease the activity of these enzymes at high concentrations.

Ashfield *et al.* (1998) investigated the effects of prolonged exposure to nonylphenol on growth and the gonado(ovo)somatic index of female juvenile rainbow trout (*oncorhynchus mykiss*). Groups of 200 fish were exposed to three concentrations of 4-nonylphenol using a flow-through system from hatch to early sexual maturity (approximately one month after hatch). Two series of experiments were conducted. In the first series, exposure to nonylphenol (nominal concentrations 1, 10 and 50  $\mu\text{g/L}$ ) was for 22 days from hatch, and monitoring of the fish was continued for a further 86 days. In the second series, exposure to nonylphenol (nominal concentrations 1, 10 and 30  $\mu\text{g/L}$ ) was for 35 days from hatch, with monitoring of fish continuing for a further 431 days. In all tests, the water had a pH of 6.5, a hardness of 12.5 mg/l as  $\text{CaCO}_3$ , a temperature of 7-13 $^\circ\text{C}$  and was continuously aerated to maintain the dissolved oxygen level. The stock nonylphenol solutions were made up in methanol/water mixture and each exposure solution had around 0.0005% methanol present (the same amount of methanol was added to the control). In the tests no significant difference was seen in total mortality between controls and treated fish. At the end of the first series of tests, fish that had been exposed to 1 and 50  $\mu\text{g/L}$  showed a statistically significant ( $\rho < 0.001$  and  $\rho < 0.01$  at the two concentrations respectively) lower body weight relative to controls (the 10  $\mu\text{g/L}$  group was not significantly different from the control group). In the second series of experiments, by day 55 the mean weights and lengths of fish exposed to 30  $\mu\text{g/L}$  were significantly lower ( $\rho < 0.05$  for weight,  $\rho < 0.01$  for length) than in the control group. The 10  $\mu\text{g/L}$  group showed no significant effect on weight at this time, but the length was significantly reduced ( $\rho < 0.05$ ) compared with controls. These differences in weight and length became more pronounced at day 84, with significantly lower weights in the 10  $\mu\text{g/L}$  ( $\rho < 0.001$ ) and 30  $\mu\text{g/L}$  ( $\rho < 0.01$ ) groups. The significantly reduced body weight seen in the 30  $\mu\text{g/L}$  group continued up until the experiment ended on day 466, but the fish exposed to 10  $\mu\text{g/L}$  showed a significantly elevated bodyweight ( $\rho < 0.05$ ) compared with controls from day 300 onwards. The fish body weight in the 1  $\mu\text{g/L}$  group was not significantly different from controls at any time during the experiment. At the end of the experiment, the ovosomatic index ( $\text{OSI} = (100 \times \text{gonad weight} / [\text{bodyweight} - \text{gonad weight}])$ ) was determined, and this was found to be significantly elevated ( $\rho < 0.05$ ) in the 30  $\mu\text{g/L}$  group. The paper concluded that significant effects on growth of the fish had occurred during the test, although the mechanism by which nonylphenol caused these effects was unclear.

## 6.2 Appendix 2: *In vivo* data on human endocrine effects (Source: EU-RAR, 2002)

Individual references are listed in the EU-RAR.

Only data from animals or *in vitro* test systems were available.

### Studies investigating estrogenic activity:

The estrogenic activity of nonylphenol has been investigated in a number of studies using either recombinant yeast, estrogen sensitive MCF-7 cells or a rodent uterotrophic assay response. None of these assays have been validated as an internationally accepted toxicity test method, although the MCF-7 and uterotrophic assays have been established for a number of years as standard assays for estrogenic activity. It should be noted that the significance to human health of estrogenic activity detected in these assays has yet to be established.

### *In vivo* systems

The estrogenic activity of nonylphenol has been assessed in several studies using an assay based upon the uterotrophic response in the rat. In the first study, five groups of immature (aged 20-22 days) female rats (six in each group) of a Wistar-derived strain received single oral gavage doses of nonylphenol in corn oil on three consecutive days (ICI, 1996). The dose levels ranged from 9.5 to 285 mg/kg/day. Vehicle and positive (estradiol benzoate 8 µg/kg, by subcutaneous route) groups were included. One day after the final dose the females were killed and the uterus was removed from each animal and weighed. Absolute uterus weight and bodyweight-related uterus weight were statistically significantly increased, in a dose-dependent manner, at levels of 47.5 mg/kg/day and above. The NOAEL was 9.5 mg/kg/day. The uterine response seen in the positive control group was much greater than that of the nonylphenol groups, although a direct comparison of potency is not possible given the differing exposure routes. Similar data from the same laboratory have also been presented in peer-review literature (Odum *et al.*, 1997). This latter report also included oral positive control groups (17β-estradiol, 10-400 µg/kg), which indicated that estradiol was about 1000 times more potent in this assay than nonylphenol.

In a similar assay, groups of ten ovariectomised female Sprague-Dawley rats were dosed once daily for three consecutive days by the oral route with ethanol/oil suspensions of nonylphenol at levels of 0 (vehicle control), 30, 100 and 300 mg/kg/day (Chemical Manufacturers Association 1997b). Positive control groups received ethynylestradiol in ethanol at levels of 10, 30 and 80 µg/kg/day according to the same dosing regimen. One day after the final dose the females were killed and the uterus was removed from each animal and weighed. Uterus weights at 300 mg/kg/day were significantly increased (1.5-fold) in comparison with the vehicle control group. A slightly greater response (a twofold increase) was seen in the 30 and 80 µg/kg/day positive control groups.

In an other uterotrophic assay, groups of three immature (aged 20-21 days) Sprague-Dawley rats each received a single intraperitoneal injection of nonylphenol at dose levels of 0, 1, 2 or 4 mg/animal (approximately 25, 50 or 100 mg/kg) (Lee and Lee, 1996). Estradiol,

administered by the same route, served as a positive control. The animals were killed 24 hours later and each uterus was removed, weighed and analysed for protein and DNA content and peroxidase (thought to be a uterotrophic marker enzyme) activity. There was a dose-dependent and statistically significantly increase in uterine weight at all levels, with associated increases in uterine protein and DNA content and uterine peroxidase activity. In further experiments, the uterotrophic activity of nonylphenol was found to be blocked by the co-administration of ICI 182,780, an estrogen antagonist, providing evidence that the effect of nonylphenol is mediated through the estrogen receptor. Also, the potency was compared with estradiol; in this assay estradiol was found to be about 1000-2000 times more potent than nonylphenol.

Overall, these *in vitro* and *in vivo* studies show that nonylphenol has estrogenic activity of a potency between three to six orders of magnitude less than that of estradiol.

### **Effects on fertility**

The effects of nonylphenol on fertility and reproductive performance have been investigated in a multigeneration study. The testicular toxicity of nonylphenol has been studied in a repeated exposure study.

The multigeneration study was comprehensive and was conducted in compliance with GLP (NTP 1997). The overall study design was based on the OECD two-generation reproduction toxicity study guideline, with an extension to include the production of an F<sub>3</sub> generation. Groups of thirty male and thirty female Sprague-Dawley rats were exposed to nonylphenol via incorporation in the diet at concentrations of 0 (control) 200, 650 or 2000 ppm over three generations. Calculated nonylphenol intakes were, respectively, about 0, 15, 50 and 160 mg/kg/day during non-reproductive phases and rising to around 0, 30, 100 and 300 mg/kg/day during lactation. Nonylphenol exposure commenced for the F<sub>0</sub> generation at about seven weeks of age and continued until study termination when the F<sub>3</sub> generations were about eight weeks old. F<sub>0</sub> animals were mated (one male with one female) within each dose group to produce the F<sub>1</sub> generation. Selected F<sub>1</sub> animals were similarly mated to produce the F<sub>2</sub> generation and selected F<sub>2</sub> animals were mated to produce the F<sub>3</sub> generation. For the F<sub>0</sub> generation and retained F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> animals, clinical signs of toxicity, bodyweight and food consumption were reported. Oestrous cycles were monitored prior to mating. At the necropsy of adult animals, sperm samples were taken (but not from the F<sub>3</sub> generation) for analysis of density, motility (using a computer-assisted sperm motion analysis system) from control and high dose group males, morphology, organ weights, and selected organs were sampled for histopathology. Additionally, testicular spermatid counts were made. Parameters assessed in the young offspring included litter size, bodyweight, survival, gross appearance, ano-genital distance, sexual development and, for animals killed at weaning, gross appearance of organs at necropsy and reproductive organ weights.

There was evidence of general toxicity in adults of all generations, seen as a reduction in bodyweight gain at 50 and 160 mg/kg/day and histopathological changes in the kidneys at all dose levels. These aspects are described in greater detail in section 4.1.2.6.1.

Considering the reproduction-related parameters, there were no adverse effects on fertility or mating performance. However, several other parameters were affected. Oestrous cycle length was increased by about 15% in the F<sub>1</sub> and F<sub>2</sub> females at 160 mg/kg/day, in comparison with controls. The timing of vaginal opening was accelerated by 1.5–seven days at 50 mg/kg/day and by three to six days at 160 mg/kg/day in females of the F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> generations. Also, absolute ovarian weights were decreased at 50 mg/kg/day in the F<sub>2</sub> generation and at 160 mg/kg/day in the F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> generations; however, no effect on ovarian weight was apparent in the F<sub>1</sub> and F<sub>3</sub> generations when analysed as an organ-to-bodyweight ratio. In males, changes in sperm endpoints were seen only in the F<sub>2</sub> generation; epididymal sperm density was decreased by about 10% at 50 and 160 mg/kg/day and spermatid count was decreased by a similar amount at 160 mg/kg/day. However, there may have been methodological problems with the epididymal sperm density measurements, because the density in all F<sub>2</sub> generation groups, including controls, was considerably greater (by about 25–40%) than reported for the F<sub>0</sub> and F<sub>1</sub> generation males; the age of each generation was similar at necropsy, so major differences in the sperm density would not be expected. To summarise the reproductive aspects of this study, fertility and mating performance were not adversely affected by nonylphenol treatment. However, there were changes, albeit relatively slight, in the oestrous cycle length, timing of vaginal opening, ovarian weight and sperm/spermatid count. The effects on the oestrous cycle were seen in both the F<sub>1</sub> and F<sub>2</sub> generations (not assessed in F<sub>3</sub> females) and the timing of vaginal opening was influenced in all three generations. This consistency provides firm evidence of a relationship with the treatment. These effects were possibly related to the estrogenicity of nonylphenol. There is some uncertainty about the relationship to nonylphenol treatment with respect to the ovarian weight reduction, because this effect was apparent after adjusting for bodyweight in only one generation and did not correlate with any histopathological changes. Nevertheless, it is compatible with the anticipated direct effects of exogenous estrogenic activity. Also, there is uncertainty regarding the cause of the apparent reduced sperm/spermatid numbers in the F<sub>2</sub> generation. It has been hypothesised that such changes could result from foetal or neonatal exposure to exogenous estrogenic activity (Sharpe and Skakkebaek, 1993), but if the hypothesised mechanism was operating, semen/testicular changes should also have occurred in the F<sub>1</sub> generation. Furthermore, the possibility of methodological problems adds to the difficulty in interpreting the sperm/spermatid count data. However, the observation of impaired male reproductive tract development in an intraperitoneal study provides supporting evidence in favour of the sperm/spermatid count changes being causally related to nonylphenol treatment. Furthermore, the intraperitoneal study indicates that a critical window of exposure for this effect is likely to be the neonatal period. Overall, this study provided evidence that nonylphenol exposure over several generations can cause minor perturbations in the reproductive system of offspring, which are compatible with the predictable or hypothesised effects of exogenous estrogenic activity. These perturbations did not cause functional changes in reproductive capacity of the rat at the dose levels tested. A clear NOAEL for these changes of 15 mg/kg/day was identified.

The testicular toxicity of nonylphenol was investigated in Sprague Dawley rats in a repeated dose study (de Jager *et al.*, 1999a). Groups of 20 male rats were dosed once daily by the oral (gavage) route at doses levels of 0 (vehicle control, cotton seed oil), 100, 250 or 400 mg/kg/day for a period of ten weeks, from the age of twelve weeks. The animals were killed at the end of the dosing period and a detailed evaluation of the reproductive organs was conducted. Testes and epididymal weight were recorded. The total epididymal sperm numbers were

determined. The testes were stored in Bouin's fixative and processed for histological examination, which included the identification of the stages of spermatogenesis present and the measurement of the seminiferous tubule diameter, lumen diameter and epithelial thickness. Three, fifteen and eighteen animals from the 100, 250 and 400 mg/kg/day groups, respectively, died during the dosing period; no further information on these deaths was presented. Clinical signs of toxicity were not reported. The bodyweight gain of surviving animals was not affected by treatment, although bodyweight gain was reduced among the decedents. In comparison with the control group, lower testicular and epididymal weight, tubule and lumen diameter and seminiferous epithelial diameter were seen in surviving animals at 250 and 400 mg/kg/day and the sperm count was reduced at 400 mg/kg/day. However, because of the very small group sizes due to mortality, little toxicological significance can be accorded to these findings. At 100 mg/kg/day, testes and epididymal weight were not affected, but tubule and lumen diameter and seminiferous epithelial diameter were statistically significantly lower than in the control group; the mean tubule diameter was reduced by 10%, but data for the other two parameters were not presented. Testicular abnormalities were identified by histopathology at both 250 and 400 mg/kg/day. In one animal at 250 mg/kg/day vacuolization and cell necrosis with sloughing of the epithelium was seen in about 40% of tubules. Both surviving animals at 400 mg/kg/day had tubular vacuolization, cell necrosis and derangement, with very few secondary spermatocytes and sperm being present.

This study provides evidence of nonylphenol-related testicular toxicity at exposure levels which also cause mortality. A LOAEL for testicular toxicity of 100 mg/kg/day can be designated. The observation of mortality at 100, 250 and 400 mg/kg/day in this gavage study contrasts with the findings of studies involving dietary administration summarised in the Repeated Dose Toxicity section (Hüls, 1989; Chemical Manufacturers Association, 1997a; NTP, 1997). This difference can probably be accounted for by the method of administration; gavage dosing is likely to produce higher peak concentrations of nonylphenol in the blood than dietary administration.

### **Developmental toxicity**

One oral rat developmental toxicity study was evaluated, as well as two studies looking specifically at the potential effects of NP on the developing male reproductive tract, one using the intraperitoneal route and one using the oral route.

The standard rat developmental toxicity study was conducted according to OECD guideline 414 and in compliance with GLP standards. Groups of timed-mated females of the Wistar strain were administered by oral gavage corn oil solutions of nonylphenol from days 6 to 15 of pregnancy at dose levels of 0, 75, 150 and 300 mg/kg/day. A further group receiving 600 mg/kg/day was terminated prematurely because many females died during the first few days of treatment. Sufficient females were allocated to the study to produce at least 21 pregnant females in each group. Surviving females were killed on day 20 of pregnancy and the foetuses were subjected to routine external, visceral and skeletal examinations.

There was clear evidence of maternal toxicity at 300 mg/kg/day, manifested as a reduction in bodyweight gain and food consumption, mortality of two females and macroscopic organ changes in the kidney (pale or irregular shape in seven mothers) or spleen (reduced size in two mothers). Similar macroscopic changes were seen occasionally at 150 mg/kg/day and at a high

incidence in females from the prematurely terminated 600 mg/kg/day group. No maternal toxicity was seen at 75 mg/kg/day. Post-implantation loss, litter size, foetal weights and incidence of both major and minor foetal abnormalities was not affected by treatment. This study provided no evidence of developmental toxicity in the rat at exposure levels which are toxic to the mother. The maternal NOAEL was 75 mg/kg/day and the foetal NOAEL was 300 mg/kg/day.

In the intraperitoneal study, the effects of nonylphenol on male reproductive tract development were investigated in neonatal Sprague-Dawley rats (Lee, 1998; additional information was obtained by personal communication with the author). Age-matched male pups were randomly allocated to either the control or treated groups. Daily doses of nonylphenol were administered by the intraperitoneal route at a dose volume of 5-10 µg/injection, for varying schedules between the day of birth (day 0) and 30 days of age. Control animals received the vehicle (dimethylsulfoxide) only, by the same route. The pups were killed at 31 days of age; terminal observations included external appearance of genital area, ano-genital distance, the presence of undescended testes, and reproductive organ weights (which were reported as bodyweight-related values). In the initial experiment, groups of at least three pups were dosed at 0, 0.08, 0.8 and 8 mg/kg/day, from birth to fifteen days of age. At 0.8 and 8 mg/kg/day there was a statistically significant, dose-dependent reduction in testes, epididymis, seminal vesicle and prostate weight; typically weights were about 15 to 25% less than found in the control group. Additionally, ano-genital distance was reduced at 8 mg/kg/day, only. Reproductive organ weights were not affected at 0.08 mg/kg/day. Next, groups of three or four pups received nonylphenol at 0 or 8 mg/kg/day, either from days 1 to 18 of age, days 6 to 24 or days 13 to 30, to see if there is a vulnerable phase of development. Reproductive organ weights were significantly reduced in the groups for which dosing commenced on day 1 or 6, but not in the group dosed from day 13. In a third experiment, the influence of the estrogen receptor antagonist, ICI 182,780, on nonylphenol-impaired reproductive organ weight development was investigated in groups of six or seven pups dosed with nonylphenol at 8 mg/kg/day from days 1 to 5 of age. The antagonist was administered by the intraperitoneal route at a dose of 0.5 mg/kg and dose volume of 5-10 µg/injection, ten minutes after the nonylphenol dose. It was found that ICI 182,780 blocked the effects of nonylphenol on organ weights. Administration of ICI 182,780 alone had no effect on reproductive organ weight. The incidence of undescended testes was reported in groups of between six and 34 pups dosed with nonylphenol at 8 mg/kg/day, days 1 to 5, days 1 to 10 or days 1 to 18; this was 33%, 55% and 62%, respectively. Undescended testes were not observed in vehicle control pups, in pups receiving a single dose of nonylphenol on day 1, or when ICI 182,780 was administered concurrently with nonylphenol.

In another study, eight male pups, selected from two litters, were dosed by the intraperitoneal route from days 1 to 15 of age with nonylphenol at 8 mg/kg/day and then reared to sexual maturity. Their fertility was assessed by serial pairing with either six or twenty untreated female rats and recording the number of females which became pregnant. Vehicle control male pups, selected from the same two litters, were used for comparison. Among the controls, pregnancies resulted from almost all pairings. In contrast, in the nonylphenol-treated group, two males were completely infertile, failing to impregnate any females; three were initially fertile, but failed to impregnate females in later pairings; two showed comparable fertility to the controls; the remaining male died near the start of the fertility trial. Necropsy findings were reported for five of the nonylphenol-treated males; all were observed with

undescended testes and/or either slight or marked testicular atrophy. There are a number of design weaknesses to this study: the group sizes were generally very small; the pups were apparently not weight-matched at the start of treatment; and the intraperitoneal route of administration, which could result in unrealistically high exposure of the reproductive organs, is of questionable relevance to the human risk assessment. Nevertheless, the consistent observation throughout the series of experiments of reduced reproductive organ weight or undescended testes, supported by observations of reduced ano-genital distance and, in animals reared to sexual maturity, reduced fertility, provides evidence that nonylphenol-exposure during the neonatal period impairs male reproductive tract development in the rat. The period of maximum vulnerability to this effect appears to be prior to the age of thirteen days. The blocking influence of the estrogen receptor antagonist ICI 182,780 suggests that the effect of nonylphenol on the male reproductive tract may be mediated through action on the estrogen receptor. However, in view of corrosive properties of nonylphenol and use of the intraperitoneal route of administration, it is possible that non-specific irritation of the undescended testes may have contributed to the observed effects. The author has stated that about 50% of the nonylphenol-treated pups had peritoneal cavity adhesions, while none were seen in control animals, which supports this hypothesis. Although adhesions were seen, there were no treatment-related clinical signs of toxicity or increased mortality. The blocking influence of ICI 182,780 may possibly have resulted from dilution of the injected nonylphenol (this alternative explanation was not tested as the study did not include a control group receiving nonylphenol followed by a vehicle-only injection). It should be noted that precise information on clinical signs, mortality and general macroscopic necropsy findings were not available from the author. No effects were seen in pups dosed at 0.08 mg/kg/day but, because of the very small numbers of animals receiving doses other than 8 mg/kg/day, information on the NOAEL and dose-response relationship can be gained from this study. Overall, because of the design weaknesses and the possibility that non-specific irritation may have contributed to the observed effects on the male reproductive tract, it is not possible to draw any firm conclusions from this study with respect to specific reproductive toxicity of relevance to humans.

In another study, the effects of nonylphenol exposure from the *in utero* period to sexual maturity were investigated in an oral (gavage) study (de Jager *et al.*, 1999b). Groups of ten mated females were dosed once daily with nonylphenol at levels of 0 (vehicle control, cotton seed oil), 100, 250 and 400 mg/kg/day from day 7 of pregnancy to weaning of their litters. Twenty F<sub>1</sub> generation males were randomly selected from each group for dosing as for the mother until ten weeks of age. The selected F<sub>1</sub> males were then killed. Testes and epididymal weight were recorded. The total epididymal sperm numbers were determined. The testes were stored in Bouin's fixative and processed for histological examination, which included the identification of the stages of spermatogenesis present and the measurement of the seminiferous tubule diameter, lumen diameter and epithelial thickness. No information was presented on maternal bodyweight, but it was observed that no females showed any physical or behavioural abnormalities. No offspring were born from the mothers receiving 400 mg/kg/day; it is not clear from the report if this was because of maternal deaths or embryonic/foetal resorption. There were no malformations or still births among the F<sub>1</sub> offspring. No physical or behavioural abnormalities were seen among the selected F<sub>1</sub> males. This contrasts with the De Jager (1999a) study conducted in adult males in which fifteen out of twenty animals died at 250 mg/kg/day. F<sub>1</sub> bodyweight gain over the course of the study was significantly reduced at both 100 and 250

mg/kg/day (by 11 and 20%, respectively), relative to the control group. F<sub>1</sub> absolute testicular and epididymal weights were less than the controls at both 100 and 250 mg/kg/day, but this effect was not evident when organ weights were expressed relative to bodyweight; the differences in absolute organ weight are thought likely to be related to the intergroup bodyweight differences. Total epididymal sperm count was reduced at 250 mg/kg/day (by 36%, relative to controls), but at 100 mg/kg/day sperm counts were similar to those of the control group. Seminiferous tubule diameter was slightly lower in both nonylphenol-treated groups (by about 10%); surprisingly, these slight differences were declared to be highly statistically significantly different from the control group. The authors also stated that the tubule lumen diameter and seminiferous epithelium thickness were highly statistically significantly less than the control group in both nonylphenol groups, but the data were not presented. Although these quantitative tubular changes were consistent with those of the De Jager (1999a) study, in the present study these may be related to the fact that testicular weight was lower in these groups. Histopathology revealed pathological changes in the testes of one F<sub>1</sub> male from the 100 mg/kg/day group; in the tubules, cell necrosis, vacuolation and sloughing of the germinal epithelium were described. However, no such histopathological abnormalities were seen at 250 mg/kg/day, so the changes outlined above cannot be attributed to nonylphenol treatment.

This study provides evidence of a reduction in sperm count at 250 mg/kg/day, a dose level which may have caused mortality, although it is not possible to state whether this is a developmental effect or a result of direct exposure of the males after weaning. It is not clear if the changes in the tubular measurements represent specific reproductive toxicity or non-specific secondary consequences of the reduction in bodyweight gain.