National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

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RIVM letter report 2022-0022
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Synopsis

An overview of the available data on reproduction toxicity of 4-nonylphenol

4-Nonylphenol is a substance that is being used to produce nonylphenol ethoxylates. These substances are used in paints, detergents and plant protection products.

At the request of the Health Council, RIVM has conducted literature research on the potential effects of 4-nonylphenol on reproduction. The Health Council will provide a recommendation for the hazard classification of 4-nonylphenol. As a starting point, the Health Council will use this literature research for an independent evaluation of the reproduction toxic properties of 4-nonylphenol, as requested by the Minister for Social Affairs and Employment.

With respect to reproduction toxicity endpoints, the literature research yielded six studies on the reproduction toxic properties of 4-nonylphenol in animals. The retrieved information was summarized.

Keywords: 4-nonylphenol, 4-n-nonylphenol, para-nonylphenol, reproduction toxicity, developmental toxicity

Publiekssamenvatting

4-Nonylfenol: overzicht van de beschikbare informatie over reproductietoxiciteit

De stof 4-nonylfenol wordt gebruikt bij de productie van nonylfenolethoxylaten. Deze stoffen zitten in verf, schoonmaakmiddelen en gewasbeschermingsmiddelen.
Het RIVM heeft in de wetenschappelijke literatuur onderzocht wat er bekend is over schadelijke effecten van 4-nonylfenol op de voortplanting.

Het RIVM deed dat in opdracht van de Gezondheidsraad. De Gezondheidsraad gaat een voorstel doen om de stof in een bepaalde 'gevaarsklasse' in te delen. Als voorbereiding daarop gebruikt de Gezondheidsraad het overzicht van het RIVM om te beoordelen of 4-nonylfenol schadelijk is voor de voortplanting. De minister van Sociale Zaken en Werkgelegenheid (SZW) heeft om dit advies gevraagd.

Het RIVM heeft zes studies over effecten van de stof op de voortplanting bij dieren gevonden. De gevonden informatie is samengevat.

Kernwoorden: 4-Nonylfenol, 4-n-nonylfenol, para-nonylfenol, reproductietoxiciteit, voortplanting, ontwikkelingstoxiciteit

Contents

Summary

RIVM performed a literature search for information on the potential reproduction toxicity of 4-nonylphenol. 4-Nonylphenol is a phenol compound with a linear nonyl chain at the para-position. It is a substance that is being used to produce nonylphenol ethoxylates, which are widely used as class of non-ionic surfactants and as a component of detergent, paints, herbicides, plant protection products.

Data on reproduction toxicity that addressed sexual function and fertility were available for 4-nonylphenol. These include two modified one-generation rat studies and a study which included an uterotrophic assay and a female pubertal onset assay in rats. Additionally, a postnatal study in rats was included. There was one study available on neurodevelopmental and behavioural effects of 4-nonylphenol. There were no human studies available for this specific nonylphenol type. A reference list summarizing literature in which related nonylphenol compounds were studied or where the type of nonylphenol was unclear, was also included in this report.

1 Introduction

The aim of current research is to identify and summarize the available data from studies with laboratory models, test animals and humans on the substance 4-nonylphenol. The focus of the current literature review will be on the reprotoxic properties of this substance.

Current RIVM-report does not include an assessment of the reported reprotoxic effects of 4-nonylphenol, nor does it provide a recommendation for classification of the substance based on the CLP-criteria (1). The Health Council of the Netherlands will use the summaries to assess the reprotoxic properties and to provide a recommendation for its classification. The assessment will be performed by the Health Council's Subcommittee on Classifying Reproduction Toxic Substances of the Dutch Expert Committee on Occupational Safety.

The literature search strategy which forms the basis of current literature overview is presented in chapter 2. In chapter 3 the substance identity of 4-nonylphenol is provided. Chapter 4 presents information on international classifications of 4-nonylphenol. Available information on monitoring (*i.e.* environmental and biological exposure monitoring) and manufacture and use is presented in chapters 5 and 6, respectively. A summary of the (toxico)kinetics of 4-nonylphenol is described in chapter 7. Chapter 8 describes an overview of the data on reproduction toxicity, subdivided into data on adverse effects on sexual function and fertility, adverse effects on development and adverse effects on or via lactation.

2 Literature search strategy

A literature search for publications on the effects on reproduction of 4-nonylphenol has been performed using ToxCenter up to December 2019 and using Embase, Pubmed and Scopus up to March 2021. Additionally, publications on (toxico)kinetics and monitoring were searched for as well. Below the literature search strategy and results are presented. In agreement with the Health Council, the current search for reproductive toxicity data is limited to 4-nonylphenol with CAS 104-40-5.

2.1 Embase

Table 1 presents the search terms and the results for the database Embase.

Table 1 Search strategy and result for Embase.

No.	Query	Results
#25	#10 OR #13 OR #17 OR #24	521
#24	#4 AND (#21 OR #23)	75
#23	#20 AND #22	140467
#22	'murine'/exp OR 'experimental animal'/exp OR 'animal experiment'/exp OR 'leporidae'/exp OR 'rat':ti,ab OR 'rats':ti,ab OR 'mouse':ti,ab OR 'mice':ti,ab OR 'hamser*':ti,ab OR 'pig*':ti,ab OR 'monkey':ti,ab OR 'rabbit':ti,ab	5383238
#21	#20 AND [humans]/lim	171969
#20	#18 OR #19	440200
#19	'metabolism':ti OR 'adme':ti,ab OR 'absorption distribution metabolism excretion':ti,ab	238236
#18	'xenobiotic metabolism'/exp OR 'metal metabolism'/mj OR 'metabolism'/mj	230284
#17	#4 AND (#14 OR #15 OR #16)	48
#16	(('environment*' OR 'human' OR 'biologic*') NEAR/3 'exposure monitor*'):ti,ab	123
#15	'bioaccessibility' OR 'bioelut*':ti,ab	2936
#14	'toxicokinetics'/exp OR 'toxicokinetic*':ti,ab	14009
#13	#4 AND (#11 OR #12)	284
#12	'pregnancy outcome*':ti,ab OR 'pregnan*':ti OR 'fertilit*':ti OR 'infertilit*':ti OR 'subfertilit*':ti OR 'fecundity':ti OR 'organogenes*':ti OR ((('differential' OR 'effect*' OR 'agent*') NEAR/3 'fertilit*'):ti,ab) OR (('breast' NEAR/3 'milk'):ti,ab) OR (('milk' NEAR/3 'secret*'):ti,ab) OR 'lactation':ti,ab	425305
#11	'fertility'/exp OR 'lactation'/exp OR 'breast milk'/exp OR 'pregnancy'/exp OR 'parameters concerning the fetus, newborn and pregnancy'/exp OR 'infertility'/exp OR 'organogenesis'/exp	1390023
#10	#4 AND #9	267

No.	Query	Results
#9	#5 OR #6 OR #7 OR #8	101942
#8	((('repro*' OR 'development*') NEAR/3 'toxic*'):ti,ab) OR 'teratogen*':ti,ab OR 'reprotox*':ti,ab OR 'embryotox*':ti,ab	40327
#7	'reproductive toxicity'/exp OR 'teratogenicity'/exp OR 'developmental toxicity'/exp OR 'fetotoxicity'/exp OR 'embryotoxicity'/exp	37491
#6	(('prenatal' OR 'maternal' OR 'paternal') NEAR/3 'expos*'):ti,ab	29007
#5	'prenatal exposure'/exp OR 'maternal exposure'/exp OR 'paternal exposure'/exp	28113
#4	#1 OR #2 OR #3	3696
#3	'p-nonylphenol':ti,ab,kw OR 'para-nonylphenol':ti,ab,kw OR '4-n-nonylphenol':ti,ab,kw OR '4-nonylphenol':ti,ab,kw	1309
#2	'104 40 5':rn	1058
#1	'nonylphenol'/exp	2414

2.2 PubMed

Table 2 presents the search terms and the results for the database Pubmed.

Table 2 Search strategy and result for Pubmed.

Search number	Query	Results
19	#8 or #11 or #14 or #18	243
18	#1 and #15 and #17	8
17	"rat"[tw] OR "rats"[tw] OR "mouse"[tw] OR "mice"[tw] OR "hamster*"[tw] OR "pig"[tw] OR "pigs"[tw] OR "monkey*"[tw] OR "rabbit*"[tw] OR "human*"[tw] OR "man"[tw] OR "men"[tw] OR "woman"[tw] OR "women"[tw] OR "child"[tw] OR "infant*"[tw] OR "newborn*"[tw] OR "fetus*"[tw] OR "neonate*"[tw]	23,026,869
15	"Metabolism"[Majr:NoExp] OR "metabolism"[ti] OR "adme"[tw] OR "absorption distribution metabolism excretion"[tw]	219,980
14	#1 and (#12 or #13)	157
13	"exposure monitor*"[tw] AND ("environment*"[tw] OR "human"[tw] OR "biologic*"[tw])	520
12	"Toxicokinetics"[Mesh] OR "Toxicological Phenomena"[Mesh] OR "toxicokinetic*"[tw] OR "bioaccessib*"[tw] OR "bioelut*"[tw]	459,980
11	#1 and (#9 or #10)	66
10	"pregnancy outcome*"[tw] or "pregnan*"[ti] OR "fertilit*"[ti] OR "differential fertilit*"[tw] OR "breast milk"[tw] OR "milk secret*"[tw] OR "lactaction"[tw]	311,655

Search number	Query	Results
9	"Fertility"[Mesh] OR "fertility"[tw] OR "Lactation"[Mesh] OR "Milk, Human"[Mesh] OR "Milk"[Mesh:NoExp] OR "Pregnancy"[Mesh:NoExp] OR "Pregnancy Outcome"[Mesh]	1,051,166
8	#1 and (#2 or #3 or #4 or #5 or #6 or #7)	85
7	"reproductive toxic*"[tw] OR "developmental toxicity"[tw] OR "fetotoxic*"[tw] OR "teratogen*"[tw] OR "reprotox*"[tw]	28,142
6	"Organogenesis"[Mesh] OR "Infertility"[Mesh]	184,308
5	"infertility"[tw] or "subfertility"[tw] or "fecundity"[tw] or "organogenesis"[tw]	120,334
4	"Teratogens"[Mesh] OR "Toxicogenetics"[Mesh]	8,642
3	"prenatal exposure"[tw] OR "maternal exposure"[tw] OR "paternal exposure"[tw]	41,706
2	"Prenatal Exposure Delayed Effects"[Mesh] OR "Maternal Exposure"[Mesh] OR "Paternal Exposure"[Mesh]	37,716
1	"4-nonylphenol"[Supplementary Concept] OR "4-nonylphenol"[tw] OR "pnonylphenol"[tw] OR "para-nonylphenol"[tw] OR "p-n-nonylphenol"[tw]	1,092

2.3 Scopus

A search was performed in Scopus using the following search terms:
((TITLE-ABS-KEY ('4-nonylphenol' OR 'p-nonylphenol' OR 'para-nonylphenol' OR 'p-n-nonylphenol' OR '4-n-nonylphenol') OR CASREGNUMBER (104-40-5))
AND ((TITLE-ABS-KEY (('prenatal' OR 'maternal' OR 'paternal') W/3 'expos*')) OR (TITLE-ABS-KEY ((('repro*' OR 'development*') W/3 'toxic*') OR 'teratogen*' OR 'reprotox*' or 'embryotox*'))
OR (TITLE-ABS-KEY ('pregnancy-outcome*' OR 'differential-fertilit*' OR ('breast' W/3 'milk') OR ('milk' W/3 'secret*') OR 'lactation'))
OR (TITLE ('pregnan*' OR 'fertilit*' OR 'subfertilit*' or 'infertilit*' or 'fecundity' or 'organogenes*'))) OR ((TITLE-ABS-KEY ('4-nonylphenol' OR 'p-nonylphenol' OR 'paranonylphenol' OR 'p-n-nonylphenol' OR '4-n-nonylphenol')
OR CASREGNUMBER (104-40-5)) AND ((TITLE-ABS-KEY ('toxicokinetic*' OR 'bioaccessib*' OR 'bioelut*' OR (('environment*' OR 'human' OR 'biologic*') W/3 'exposure-monitor*'))) OR (TITLE-ABS-KEY ('adme' OR 'absorption-distribution-metabolism-excretion') OR TITLE ('metabolism')))
AND (TITLE-ABS-KEY ('rat' OR 'rats' OR 'mouse' OR 'mice' OR 'hamster*' OR 'pig*' OR 'monkey*' OR 'rabbit*' OR 'human*' OR 'man' OR 'men' OR 'woman' OR 'women' OR 'child*' OR 'infant*' OR 'newborn*' OR 'fetus*' OR 'neonate*'))

This resulted in 129 document

2.4 Toxcenter

Table 3 presents the search terms and the results for the database Toxcenter.

Table 3 Search strategy and result for Toxcenter.

Query	Search terms	Number of records
L1	SEA 104-40-5	3,578
L2	SEA (PRENATAL OR MATERNAL OR PATERNAL)(3W)EXPOS?	54,947
L3	SEA (REPRO? OR DEVELOPMENT?)(3W)TOXIC? OR TERATOGEN? OR REPROTOX?	11,4511
L4	SEA PREGNANCY-OUTCOME? OR DIFFERENTIAL FERTILIT? OR BREAST(3W)M ILK OR MILK(3W)SECRET? OR LACTATION	43,596
L5	SEA (PREGNAN? OR FERTILIT?)/TI	81,729
L6	SEA TOXICOKINETIC? OR BIOACCESSIB? OR BIOELUT? OR (ENVIRONM ENT? OR HUMAN OR BIOLOGIC?)(3W)EXPOSURE MONITOR?	27,055
L7	SEA ADME OR ABSORPTION DISTRIBUTION METABOLISM EXCRETION OR METABOLISM/TI	136,805
L8	SEA L1 AND (L2 OR L3 OR L4 OR L5)	143
L9	SEA L1 AND (L6 OR L7)	60
L10	SEA L9/HUMANI	2
L11	SEA L8 OR L10	145

2.5 Other sources

The REACH registration dossier of linear 4-nonylphenol (publicly available on ECHA website) was consulted[1]. Other secondary sources were consulted as well. These included e.g. IARC, SCOEL, WHO, IPCS, ATSDR, DFG; primarily consulted via echemportal[2]. RIVM-reports and evaluations and the RIVM-website 'Risico's van stoffen'[3] were consulted as well. Of the secondary sources, the European Union Risk Assessment Report (2) and WHO/IPCS Integrated Risk Assessment Report (3) of 4-nonylphenol were most informative.

2.6 Overall evaluation of results literature search

The obtained records were evaluated, duplicates were removed, and records were included if considered relevant based on title and abstract. Publications cited in the selected publications, but not selected during the primary search, were reviewed if considered appropriate. With respect to human health endpoints evaluated in current report (i.e. reproductive toxicity), this resulted in six studies.
Although the literature search was limited to 4-nonylphenol (CAS# 104-40-5), the search also yielded literature on other types of nonylphenol. An overview of the literature concerning branched 4-nonylphenol (CAS# 84852-15-3), para-, ortho- or meta-nonylphenol (CAS# 25154-52-3), 4-nonylphenol without specification of the CAS-number, or nonylphenol without specification of the CAS-number, is given in chapter 10 (annexes 10.1-10.4).

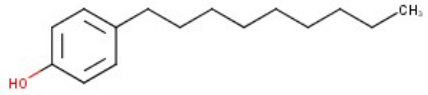
[1] https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/17497
[2] https://www.echemportal.org
[3] https://rvs.rivm.nl

3 Substance identification

3.1 Name and other identifiers of the substance

The identity of 4-nonylphenol is presented in Table 4 below. This is a phenol compound with a linear nonyl chain at the para-position as also shown in the table below.
It is noted that there are also related substances, i.e. 1) CAS 84852-15-3, a phenol compound with a branched nonyl chain at the para-position, 2) CAS 25154-52-3, a phenol compound with a nonyl chain which can be placed at the para-, ortho- or meta-position.

Table 4 Substance identity and information related to molecular and structural formula of 4-nonylphenol.

Name(s) in the IUPAC nomenclature or other international chemical name(s)	4-nonylphenol
Other names (usual name, trade name, abbreviation)	p-nonylphenol, 4-n-nonylphenol, p-n-nonylphenol
ISO common name (if available and appropriate)	N/A
EC/EINECS number (if available and appropriate)	203-199-4
EC name (if available and appropriate)	p-nonylphenol
CAS number	104-40-5
Other identity code (if available)	N/A
Molecular formula	$C_{15}H_{24}O$
Structural formula	
SMILES notation (if available)	CCCCCCCCCC1=CC=C(O)C=C1
Molecular weight or molecular weight range	220.35 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	N/A
Description of the manufacturing process and identity of the source (for UVBC substances only)	N/A
Degree of purity (%) (if relevant for the entry in Annex VI)	N/A

3.2 Physicochemical properties

The physicochemical properties of 4-nonylphenol are presented in Table 5 below.

Table 5 Summary of physicochemical properties

Properties	Value (4-nonylphenol; CAS 104-40-5)	Ref.
State of the substance at normal temperature and pressure	viscous liquid	1
Melting/freezing point	42 °C	1
Boiling point	293 - 297 °C	1
Relative density	0.937 g/cm³ (20 °C)	1
Surface tension	No data available	
Water solubility	7 mg/L (25 °C)	1
Part ition coefficient n-octanol/water	5.76	1
Flash point	166 °C	1
Flammability	No data available	
Explosive properties	No data available	
Self-ignition temperature	No data available	
Oxidising properties	No data available	
Granulometry	No data available	
Stability in organic solvents and identity of relevant degradation products	No data available	
Dissociation constant (pKa)	No data available	
Viscosity	No data available	

(1) https://echa.europa.eu/nl/registration-dossier/-/registered-dossier/17497

4 International classifications

4.1 European Commission

4-Nonylphenol (CAS 104-40-5) has currently no harmonised classification in Annex VI of the CLP-Regulation (EC) 1272/2008.

4.2 The Health Council

4-Nonylphenol (CAS 104-40-5) has not previously been evaluated by the Health Council of the Netherlands.

4.3 Other countries

4-Nonylphenol (CAS 104-40-5) has the following classification in Japan[4]:

- Aquatic Acute 1 (H400: Very toxic to aquatic life)
- Aquatic Chronic 1 (H410: Very toxic to aquatic life with long lasting effects)

4-Nonylphenol (CAS 104-40-5) has the following classification in Australia[5]:

- Acute Tox. 4 (H302: Harmful if swallowed)
- Skin Corr. 1B (H314: Causes severe skin burns and eye damage)
- Repr. 2 (H361fd: Suspected of damaging fertility. Suspected of damaging the unborn child)

In Germany, 4-nonylphenol (CAS 104-40-5) is not included in the list of additional CMR substances in the context of worker protection.[6]

No evaluation by the International Agency for Research on Cancer (IARC) was found.

In the state of California, 4-nonylphenol (CAS 104-40-5) is not included in the list of chemicals known to the state to cause cancer or reproductive toxicity.[7] 4-Nonylphenol is not included in the Report on Carcinogens (14th edition)[8], and also not in the NIOSH carcinogen list[9].

[4] https://www.nite.go.jp/chem/english/ghs/19-moe-0142e.html
[5] http://hcis.safeworkaustralia.gov.au/HazardousChemical/Details?chemicalID=5470
[6] https://www.baua.de/DE/Angebote/Rechtstexte-und-Technische-Regeln/Regelwerk/TRGS/pdf/TRGS-905.pdf?__blob=publicationFile
[7] https://oehha.ca.gov/media/downloads/proposition-65//p65list091319.pdf
8 https://ntp.niehs.nih.gov/whatwestudy/assessments/cancer/roc/index.html
[9] https://www.cdc.gov/niosh/topics/cancer/npotocca.html

5 Monitoring

5.1 Environmental exposure monitoring

Because of the low solubility, 4-nonylphenol accumulates in environmental matrices such as soils and sediments (4) and has been detected in almost all environmental water matrices, including drinking water (5). Due to its lipophilic properties and long half-life, nonylphenol is ubiquitous in the food chain, including fish, animal tissues, milk and cereals (5).

No analytical methods for 4-nonylphenol are presented in the NIOSH Manual of Analytical Methods (5th Edition)[10].

Both gas chromatography - mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) are described as methods to analyse 4-nonylphenol in air samples. Rudel et al. used a GC-MS analytical method, which requires derivatization (with N,O-bis-(trimethylsilyl) trifluoroacetamide) of the extract prior to analysis (6). Inoue et al. described a method for determining 4-nonylphenol in air samples by using stable isotope dilution techniques and LC-MS (7). The isotope 4-(1-methyl)- octylphenol-d_5 was used as a standard for 4-nonylphenol.

5.2 Biological exposure monitoring

With respect to biological exposure monitoring, no analytical methods for 4-nonylphenol are presented in the NIOSH Manual of Analytical Methods (5th edition).
The most common method for 4-nonylphenol analysis is by using GC-MS and a two-dimensional application with a GCxGC- time-of-flight (ToF)-MS (8).
Nonylphenols can be separated by HPLC technics (9).
An example of GC-MS measurement of 4-nonylphenol is given by Shekhar et al. (10). Also, a combination of solid phase extraction -high performance liquid chromatography–tandem mass spectrometry (SPE-HPLC-MSMS) has been used to measure 4-nonylphenol in blood, performed by the Organisation for Applied Scientific Research (TNO) in the Netherlands (11).
Measurements in urine have been performed by Kim et al. (12) and Park et al. (13) using liquid-liquid extraction and GC-MS.

[10] https://www.cdc.gov/niosh/nmam/default.html

6 Manufacture and uses

Nonylphenols are produced industrially by the acid-catalyzed alkylation of phenol with a mixture of nonenes, leading to a mixture of nonylphenols with differentially branched nonyl-sidechains (14, 15). Nonylphenol is mainly used as an intermediate in the manufacture of the non-ionic surfactants nonylphenol ethoxylates. Those ethoxylates were used in detergents, paints, plant protection products, personal care products, and plastics. Nonylphenol ethoxylates reach sewage treatment works where they are degraded to nonylphenol (16).

EU member states and industry have voluntarily agreed to phase out nonylphenol ethoxylates in all detergent applications by the year 2000 (2). Also the US-EPA has agreed with companies to phase out nonylphenol ethoxylates in all liquid and powder detergent formulations by 2015.[11]
Furthermore, ECHA's Member State Committee agreed in 2013 on the identification of nonylphenol ethoxylates as a substance of very high concern, based on its endocrine disrupting properties. Subsequently, the substance is included in the authorisation list (Annex XIV of the REACH Regulation (EC) No 1907/2006) in 2017, meaning that authorisation is required to be allowed to use the substance.[12] Additionally, the use of nonylphenol ethoxylates is restricted in textile articles (Annex XVII of the REACH Regulation (EC) No 1907/2006) in 2021.[13]

According to the REACH registration dossier, the total manufacturing and or import of 4-nonylphenol in the EU is 1-10 tonnes per year. The uses of 4-nonylphenol include formulation into a mixture, which results in the following products: coatings and paints, thinners, paint removes, fillers, putties, plasters, modelling clay, ink and toner. Described industrial and professional uses of 4-nonylphenol are use in coatings, paints, thinners and paint removers.

[11] https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-management-nonylphenol-and-nonylphenol-ethoxylates
[12] https://echa.europa.eu/documents/10162/a017d68e-19ca-fa1b-2659-517986470f43
[13] https://echa.europa.eu/documents/10162/7dcd73a4-e80d-47c5-ba0a-a5f4361bf4b1

7 Toxicokinetics

In this section, a short summary is provided based on the WHO 2002 (3) and EU-RAR 1998 (2) reports and the RAC/SEAC background document on nonylphenol ethoxylates (17). Additional individual studies are included as well.
It is noted that the consulted literature for this chapter comprised of studies performed with linear 4-nonylphenol (CAS 104-40-5), branched 4-nonylphenol (CAS 84852-15-3) or 4-nonylphenol which was not further specified. For this reason, there is some uncertainty with respect to the specific toxicokinetics of linear 4-nonylphenol.

7.1 Human data

The toxicokinetics of nonylphenol has been studied in two male volunteers by Muller et al. (1998) as a single oral dose of 66 µg/kg bw or a single intravenous dose of 14 µg/kg bw of 4-n-nonylphenol (18). Time to peak concentration in the blood after oral exposure was 1 hour with conjugated nonylphenol levels (sulphate or glucuronide) of 86 ng/g blood. This was 100-fold higher than the unconjugated form which is indicative of extensive first-pass metabolism. After intravenous and oral application, the elimination half-life of the parent compound from the blood was 2–3 h. The bioavailability after oral application was about 20%. A distribution volume of 2800 L was calculated, suggesting distribution to lipid. About 10% of the oral dose was excreted via urine either in the conjugated or unconjugated form, whereas less than 1% was excreted via the faeces up to 56 hours after administration, indicating that the substance was quantitatively absorbed in the gastrointestinal tract.
A study examining postmortem adipose tissue, found that measured tissue concentrations were in line with the background concentrations observed in the negative controls (18).
A study in 100 Swedish women found that 85% of the nonylphenol present in blood was free nonylphenol. A relatively high number of young women had low but detectable levels of nonylphenol in their bloodstream (11).

Dermal absorption and penetration was studied in vitro by Monteiro-Riviere et al. (19). The authors found absorption of 0.1% and penetration of 4% of the administered dose with the majority being distributed to the stratum corneum (19).

Several studies have also addressed placental transfer and foetal exposure to nonylphenol and these show that nonylphenol can cross the placental barrier. Lin et al. (2008, abstract only) quantified 4-nonylphenol levels in cord blood of on average 1.12 mg/L versus 1.51 mg/L in maternal blood (20). Another study found in maternal blood and in amniotic fluid of 53 women during caesarean section at term comparable levels of 9.38 ng/mL 8.44 ng/mL, respectively (10).
Maternal to foetal transfer of linear 4-nonylphenol (CAS# 104-40-5) has also been studied in a human placenta perfusion study (21).
Nonylphenol levels rapidly declined in the maternal circulation and after

1h nonylphenol entered the foetal circulation. The authors suggest that this indicates the formation of an intermediary metabolite in the placenta or high placental tissue binding. After 3 hours, the maternal-to-foetal transfer rate was 0.8 (21).

One study quantified nonylphenol exposure in decidua and chorionic villi samples in early first trimester pregnancies. The geometric mean concentrations where higher in chorionic villus samples as compared to maternal decidual samples (5.33 ng/g dry weight versus 3.27 ng/g dry weight) indicating placental exposure and likely also foetal exposure, but actual blood or amniotic fluid concentrations were not measured (22).

7.2 Animal data

In a study of Green et al., it was demonstrated that administered ^{14}C-labelled para-nonylphenol is rapidly absorbed (up to 80%), after male and female CD rats received 10 or 100 mg/kg bw single oral doses, 10 mg/kg bw single i.v. doses, or repeated daily oral doses of 10 mg/kg bw for up to 14 days of ^{14}C-nonylphenol. Excretion was largely complete within 24h of dosing. Following absorption, nonylphenol was metabolised in the liver, with the majority of the metabolites excreted in bile, mainly as glucuronide conjugates (23).

In a study of Knaak et al., ^{14}C-labelled nonylphenol was administered to rats (24). About 70% of the administered radioactivity was excreted via faeces, and 20% via the urine. Most of the radioactivity was excreted within 4 days after administration (24).
Xiao et al., studied the toxicokinetic parameters of 4-nonylphenol in male Wistar rats (25). After a single oral administration of 4-nonylphenol (200 mg/kg bw), peak serum concentrations were found to be approximately 4 µg/mL and were reached after 1-2 hours. Serum half-life was approximately 3 hours.

Lee et al. studied *in vitro* metabolism of ^{14}C-labelled nonylphenol mixture containing multiple nonylphenol isomers in rat liver microsomes and human microsomes derived from a lymphoblast cell line (26). Nonylphenol was metabolised by CYP450 enzymes and metabolism by CYP2B isoenzymes was observed in both rat and human microsomes (26). Based on results in Wistar rat (27), 4-nonylphenol is mainly metabolised through oxidation of the alkyl side chain (prior or after the conjugation of the phenol group to sulphate or to glucuronic acid). The second major metabolic pathway of 4-nonylphenol is the hydroxylation of the alkyl side chain, followed by the glucuronidation of the phenol moiety. The corresponding metabolite is mainly excreted by faecal route (27).

Metabolism and excretion were determined using an in vivo perfused rat liver model with 4-nonylphenol (0.025 or 0.05 mM) (28). About 800 to 1000 nmol of injected nonylphenol could be conjugated as glucuronide within 1 h and most of the glucuronide and free nonylphenol remained in the liver (28).
Distribution and elimination were also investigated in everted intestinal tissue of SD rats (29). 4-Nonylphenol was readily absorbed and glucuronidated within intestinal tissue of Sprague-Dawley rats. This was

confirmed in microsomes prepared from intestinal tissue which showed strong glucuronidation activity. However, both nonylphenol and nonylphenol-glucuronide were not excreted from intestinal tissue within 10 h. Additionally, orally administered nonylphenol was persistent in the gastrointestinal tissue (29).

A metabolic saturation in female rats was suggested after given a 100 mg/kg bw dose (oral), after which unchanged NP was found only in bile and urine (23). Following repeated dosing, steady state was reached within 7 days (23). According to Green et al., this suggest that the oestrogen-like effects observed in toxicity studies with female rats at oral nonylphenol doses of approximately 50 mg/kg bw/d and greater are a result of the increased bioavailability of nonylphenol which occurs following metabolic saturation (23).

Differences in toxicokinetics between male and female rats were also described. 4-Nonylphenol was more rapidly eliminated from the blood in female rats compared to male rats, and oral absorption to blood was faster in male rats than that in female rats. Distribution and accumulation of 4-nonylphenol into tissues were greater in male rats than those in female rats (30).
4-Nonylphenol was more extensively metabolised in male rats, with a number of metabolites present in urine, bile, and faecal extracts that were not seen in female rats (23). Furthermore, females showed an increased half-life in the plasma in parallel with increasing concentrations, whereas in males the half-life in plasma was not affected by increasing doses (23). According to Green et al., this difference suggests that the capacity of the female rat to metabolize and excrete branched 4-nonylphenol is similar or even slightly greater than males at low doses and is significantly lower than that of males at high doses (23).

The sex difference was also seen in comparisons of metabolite profiles in urine, bile and faecal extracts (23). The intestinal reabsorption of 4-nonylphenol residues and their possible entero-hepatic cycling could be different in male and female rats (27). It appeared that sulfoconjugation was more pronounced in male than in female rats (27). Following a 100 mg/kg bw dose, significant amounts of nonylphenol itself were present in female but not male bile. These findings again suggest that the capacity of the liver to form this glucuronide is saturated at the higher dose in females (23). The nonylphenol glucuronide, the major metabolite in female rats, was not present in male rat urine, although it was present in bile. These profiles suggest further metabolism of NP (either on the aromatic ring or the nonyl side chain) in males that does not occur in females (23).

Fu et al. studied toxicokinetics and tissue distribution in female Sprague-Dawley rats after repeated nonylphenol exposure (17 weeks) at three dose levels (50-, 500-, 10.000 µg/kg bw/day) (31). Pharmacokinetic parameters were calculated through a two extravascular compartmental model. Peak serum concentrations were reached after approximately 1 week and amounted to 2.58-2.74 ng/mL. Nonylphenol was distributed widely with the highest concentrations in the uterus, adipose and brain. The amount of nonylphenol in the uterus increased over time to

approximately 100 µg/g tissue in the mid and high dose group, while the brain levels decreased over time (31).

Nonylphenol is distributed widely in rats but the amount of radioactivity detected in the tissues was very low (0.4%) after a single dose of radiolabelled nonylphenol (32). Distribution and accumulation into tissues were greater in male rats (23). Accumulation of nonylphenol was found in adipose, ovaries, liver and kidneys, uterus and brain (33). Detection in the brain indicated that nonylphenol could cross the blood-brain barrier (33). Xiao et al. also found that a small amount was distributed to the testis (25). Placental transfer was observed in another study in rats and results of this study indicate that nonylphenol could be detected in the foetal brain as well (34).

8 Toxicity for reproduction

8.1 Adverse effects on sexual function and fertility

8.1.1 *Animal studies*

Table 6 summarizes the studies of 4-nonylphenol in experimental animals focusing on adverse effects on sexual function and fertility. Only results that were statistically significant ($P<0.05$) were included in the table.

Table 6 Summary table of animal studies on adverse effects on sexual function and fertility

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on reproductive organs or reproduction	Remarks
Oral exposure						
Hossaini et al., 2001 (35)	Rat, Wistar, female, number unknown After delivery, the numbers of male and female offspring were adjusted to 10 per litter by reducing the number of females only.	Modified one-generation study. Daily exposure at gestational days 11-18 Effect parameters: Body weight gain in pregnant dams, post-mortem examination and organ weights of dams and offspring, litter size, pup birth weight, pup body weight, sperm motility, sperm count, hormone levels, biochemical parameters. Anogenital distance at PND 1, 5, 10, 15, and 21 (males + females), age at preputial separation, age at vaginal opening. Statistical analysis: - Mixed linear models (for terminal body weight, hormone levels, testis and liver weight) - Two-tailed t-test was used for comparison to control group.	Test item: 4-nonylphenol (CAS# 104-40-5, purity: 99.5%) Route of exposure: oral gavage Exposure levels: 0 (vehicle), 3, 15, or 75 mg/kg bw/day Negative control: Vehicle (peanut oil) Positive control: Diethylstilbesterol (DES); 30 µg/kg bw/day	No parental toxicity. No effect on body weights in pups. 75 mg/kg bw/day Decreased liver weight at PND 21 in males 15 mg/kg bw/day Decreased liver weight at PND 11 and 21 in males	75 mg/kg bw/day Decreased absolute weight of the right epididymis 15 mg/kg bw/day Decreased absolute weight of the right epididymis	No guideline study. No information on GLP.

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on reproductive organs or reproduction	Remarks
		- Other parameters were analysed using a one-way ANOVA with the Dunnett's t-test for comparison to control group.				
Dalgaard et al., 2002 (36)	Rat, Wistar, female, number of animals per dose group unspecified. Evaluation of male offspring, 11 days old. n= 7/dose group; 1-2 siblings per litter	Modified one-generation study. Daily exposure during gestational day 11-18. Effect parameters: Testis weight and histopathology, diameter and length of seminiferous tubules, number of Sertoli cells. Statistical analysis: One-way ANOVA on seminiferous tube length and diameter and number of Sertoli cells, litter was used as a random independent variable	Test item: 4-n-nonylphenol (CAS# 104–40–5, purity 99.5%) Route of exposure: oral gavage Exposure level: 75 mg/kg bw/day Negative control: Vehicle (peanut oil) Positive control: DES (30 µg/kg bw/day)	No data reported on general toxicity.	No statistically significant effects observed upon treatment with 4-nonylphenol.	No guideline study. No information on GLP.
Kim et al., 2002 (37)	Rat, Sprague–Dawley, females.	Female pubertal onset assay 21-d old females were treated daily for 20 days.	Test item: 4-nonylphenol (Cas# 104-40-5); a mixture of	100 mg/kg bw/day	100 mg/kg bw/day - Decreased absolute and relative	No guideline study. No

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on reproductive organs or reproduction	Remarks
	n=10/group; 21 days old	Analysed parameters: Clinical signs and body weight, organ weights (liver, heart, kidney, thyroid, thymus, pituitary glands, and adrenal glands), vaginal opening and hormonal measurements. Statistical analysis - Nonparametric analysis of variance was applied. -Organ weights were analysed using analysis of covariance (ANCOVA) with the body weight at necropsy as a covariate. - Dunnett's test was used to compare control versus treatment groups.	branched side-chains containing a minimum of 92% *p*-isomers [14] Route of exposure: oral gavage Exposure levels: 0, 10, 50 and 100 mg/kg bw/day Negative control: vehicle (not specified) Positive control: DES (0.2, 1 and 5 µg/kg bw/day)	Decreased kidney weight and pituitary weight.	combined ovaries weight. - Decreased absolute and relative uterus weight . - Accelerated vaginal opening with 6.1 days (26.3 *vs.* 32.4 days in controls) - Decreased serum T4 levels - Increased number of days in dioestrus <u>50 mg/kg bw/day</u> - Accelerated vaginal opening with 0.9 days (31.5 *vs.* 32.4 days) - Increased number of days in dioestrus <u>10 mg/kg bw/day</u> no effects	information on GLP.

[14] This was interpreted as linear 4-nonylphenol (CAS# 104-40-5) with a purity larger than 92%.

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on reproductive organs or reproduction	Remarks
Subcutaneous exposure						
Kim et al., 2002 (37)	Rat, Sprague–Dawley, females. n=10/group; 20 days old	Uterotrophic assay: treatment of immature rats on PND 20 by subcutaneous injection, once per day for three consecutive days (20, 21, and 22 days of age). Analysed parameters: Uterine, vagina and ovary weights. Statistical analysis -Organ weights were analysed using analysis of covariance (ANCOVA) with the body weight at necropsy as a covariate. - Dunnett's test was used to compare control versus treatment groups.	Test item: 4-nonylphenol (Cas# 104-40-5); a mixture of branched side-chains containing a minimum of 92% *p*-isomers[15] Route of exposure: subcutaneous injection Exposure levels: 0, 10, 25, 50, 100 and 200 mg/kg bw/day Negative control: vehicle (not specified) Positive control: DES (0.2, 1 and 5 µg/kg bw/day)	<u>200 mg/kg bw/day</u> Decreased body weight	<u>200 mg/kg bw/day:</u> - Increased wet uterine weight - Increased vaginal weight - Decreased ovarian weight <u>100 mg/kg bw/day</u> - Increased wet uterine weight - Increased vaginal weight - Decreased ovarian weight	Procedure was based on the OECD validation protocol (1999). No information on GLP.

[15] This was interpreted as linear 4-nonylphenol (CAS# 104-40-5) with a purity larger than 92%.

Reference	Species	Experimental period and design	Dose and route	General toxicity	Effects on reproductive organs or reproduction	Remarks
Willoughby 2005 (38)	Rat, Sprague–Dawley, females. n=6-8 per treatment group; 1 day old females.	Postnatal exposure study. Exposure period: PND1-10 Parameters: day of vaginal opening, ovulation, prepubertal LH levels, LH response to oestradiol, oestrous cyclicity, and ovarian histology. Statistical Analysis - T-test for comparisons between two groups. - One-way or two-way ANOVA for comparisons between multiple groups. Post-hoc test: Student–Newmann–Keuls test.	Test item: 4-nonylphenol; no data on purity Route of exposure: injected subcutaneously Exposure levels: 0, 5 and 50 mg/kg bw/day Negative control: vehicle (corn oil)	No effects observed on body weight. No other general toxicity was reported.	No significant effects observed upon treatment with 4-nonylphenol.	No guideline study. No information on GLP. Although the authors do not specify the CAS-number of the test item, the supplier and catalogue number are specified and correspond to CAS #104-40-5.

Modified one-generation study in rats (Hossaini et al., 2001)
Male and female reproduction parameters were studied in a modified one-generation study by Hossaini et al. (35). Pregnant dams were exposed to 4-nonylphenol at a dose of 0, 3, 15 or 75 mg/kg bw/day by oral gavage at gestational days 11-18. Body weight was monitored during gestation and after delivery.
In male offspring, anogenital distance, age at preputial separation, total sperm count, sperm motility and blood biochemical parameters and hormone levels were assessed. Weight of the liver, testis, adrenals, epididymis, seminal vesicles and ventral prostrate in adult males were recorded. In female offspring, anogenital distance and age at vaginal opening were assessed.
No deaths or other signs of parental toxicity were observed in the dams exposed to 4-nonylphenol. There was no effect of 4-nonylphenol on the number of resorptions in dams.
Table 7 presents the results of this study. No effects on pup body weights were observed. The absolute liver weight on PND 21 was reduced in the 15 mg/kg bw/day and 75 mg/kg bw/day dose group and on PND 11 in the 15 mg/kg bw/day dose group only. The weight of the right epididymis was reduced at 15 and 75 mg/kg bw/day 4-nonylphenol ($P<0.05$).

Table 7 Summary of effects of 4-nonylphenol on fertility parameters as described by Hossaini et al., 2001 (35)

Outcome parameter	**Control**	**Nonylphenol 3 mg/kg**	**Nonylphenol 15 mg/kg**	**Nonylphenol 75 mg/kg**	**Positive control DES 30 µg/kg**
Body weight (g)	380 ± 26	390 ± 25	368 ± 41	390 ± 54	339 ± 21*
Liver (g)	11 ± 1	11 ± 1	11 ± 1	11 ± 2	9.5 ± 0.9
Adrenals (mg)	60 ± 10	65 ± 10	59 ± 8	59 ± 18	57 ± 7
Seminal vesicles (g)	1.27 ± 0.17	1.17 ± 0.18	1.20 ± 0.17	1.11 ± 0.19	1.16 ± 0.21
Prostate (mg)	770 ± 180	764 ± 100	719 ± 110	749 ± 120	743 ± 110
Right testis (g)	1.78 ± 0.11	1.75 ± 0.14	1.68 ± 0.95	1.57 ± 0.44	1.69 ± 0.15
Left testis (g)	1.78 ± 0.11	1.77 ± 0.11	1.70 ± 0.11	1.59 ± 0.45	1.69 ± 0.14
Right epididymis (mg)	606 ± 55	575 ± 42	544 ± 36*	525 ± 96*	538 ± 24*
Left epididymis (mg)	608 ± 50	580 ± 35	559 ± 56	536 ± 96	541 ± 18
Motile sperm (%)	83 ± 8	77 ± 9	77 ± 6	84 ± 5	72 ± 12*
Progressive sperm (%)	47 ± 17	55 ± 12	43± 21	55 ± 13	40 ± 15
Straight line velocity (µm/s)	114 ± 10	120 ± 10	114 ± 23	118 ± 10	112 ± 17
Path average velocity (µm/s)	169 ± 13	172 ± 15	167 ± 27	176 ± 10	158 ± 20
Amplitude of lateral head displacement (µm)	14 ± 3	15 ± 3	15 ± 4	13 ± 2	13 ± 3
Straightness	67 ± 3	69 ± 2	68 ± 4	67 ± 4	69 ± 4
Sperm/g cauda $*10^6$	478 ± 92	485 ± 119	476 ± 63	426 ± 111	465 ± 102
Age at preputial separation (days)	51 ± 2	51 ± 4	51 ± 1	51 ± 4	52 ± 4
Age at vaginal opening (days)	36 ± 2	36 ± 2	37 ± 2	38 ± 3	37 ± 2

Values are mean ± SD.
Each group contained 9 to 11 animals for organ weights and sperm parameters. Total no. of animals: Vaginal opening: 101, preputial separation: 82.
* Significantly different from control ($P < 0.05$).

Modified one-generation study in rats (Dalgaard et al., 2002)
This study is presented as another part of the study of Hossaini et al. (2001) (35). Male reproduction parameters were studied in 11-day-old male Wistar rats after prenatal exposure to 4-nonylphenol (36). Pregnant dams were exposed to 75 mg/kg bw/day 4-nonylphenol during gestational day 11-18 by oral gavage. Male offspring (1-2 siblings per litter, 7 animals/group) was examined for testis weight and

histopathology, the diameter and length of seminiferous tubules, and the number of Sertoli cells.
No differences in testis weight, histopathology, the length or diameter of the seminiferous tubules, or number of Sertoli cells were observed in the 4-nonylphenol exposed group when compared to the control group. Results were given in figures; no absolute number were presented.

Uterotrophic assay in rats (Kim et al., 2002)
The estrogenic activity of 4-nonylphenol was examined in an uterotrophic assay and in a female pubertal onset assay with female Sprague–Dawley rats (37).
In the uterotrophic assay, immature rats were treated with 0, 10, 25, 50, 100 and 200 mg/kg bw/day 4-nonylphenol by subcutaneous injection. Treatment started on PND 20 and was performed once per day for three consecutive days. After treatment, uterine, vagina and ovary weights were measured.
Table 8 present the results of this uterotrophic assay. There was no mortality, no evidence of overt toxicity, and no treatment-related clinical signs during the study period. Decreased body weight was seen in the highest dose group. The wet uterine weight and the vaginal weight were increased at 100 and 200 mg/kg bw/day groups ($P<0.05$) and the ovarian weight was decreased in the same dose groups ($P<0.05$).

Table 8 The effects of 4-nonylphenol on body and organ weights in a rat uterotrophic assay as described by Kim et al., 2002 (39)

Outcome parameter	Control	Nonylphenol 10 mg/kg	Nonylphenol 25 mg/kg	Nonylphenol 50 mg/kg	Nonylphenol 100 mg/kg	Nonylphenol 200 mg/kg	DES 0.2 µg/kg	DES 1 µg/kg
Initial body weight (g)[a]	49.0 ± 1.8	49.7 ± 2.6	49.6 ± 2.4	48.9 ± 2.5	50.2 ± 2.6	49.1 ± 2.7	49.5 ± 2.2	49.1 ± 1.6
Final body weight (g)[b]	62.3 ± 2.3	62.2 ± 3.1	61.4 ± 2.6	61.1 ± 4.6	61.7 ± 3.8	54.8 ± 2.9*	61.8 ± 3.2	62.6 ± 2.6
Uterus (mg)	23.9 ± 4.0	28.2 ± 6.3	29.3 ± 4.3	31.7 ± 5.8	57.3 ± 9.9*	76.2 ± 9.1*	42.7 ± 7.4*	171.9 ± 7.5*
Vagina (mg)	34.4 ± 8.3	31.5 ± 8.2	34.1 ± 6.1	34.6 ± 7.4	50.2 ± 6.3*	61.0 ± 9.2*	39.3 ± 6.7	71.6 ± 8.8*
Combined ovaries (mg)	24.5 ± 3.1	20.3 ± 7.4	20.2 ± 6.7	17.6 ± 6.4	17.1 ± 3.9*	16.4 ± 6.5*	19.5 ± 6.4	15.7 ± 4.2*
Liver (g)	2.51 ± 0.14	2.60 ± 0.22	2.66 ± 0.14	2.59 ± 0.22	2.56 ± 0.27	2.49 ± 0.18	2.51 ± 0.15	2.52 ± 0.16

Mean ± S.D. (n=10 animals per treatment group).
[a] Body weight on the first day of treatment (21 days of age).
[b] Body weight on the day of necropsy (41 days of age).
* Significantly different from control by Dunnett's test (P< 0.05).

Female pubertal onset assay in rats (Kim et al., 2002)
In the female pubertal onset assay, 21-day old females were treated with 0, 10, 50 and 100 mg/kg bw/day 4-nonylphenol by oral gavage daily for 20 days, starting at day 21. Organ weights, vaginal opening and several hormone levels were measured.
Tables 9-12 present an overview of the results of this assay. In this female pubertal onset assay, a decrease in kidney weight and pituitary weight was observed at 100 mg/kg bw/day. At 100 mg/kg bw/day, the combined ovaries weight (absolute and relative) and uterus weight (absolute and relative) was decreased, there was an accelerated vaginal opening (6.1 days compared to controls), the serum T4 levels were decreased and the number of days in dioestrus was increased (all $P<0.05$). An accelerated vaginal opening (0.9 days) and increased number of days in dioestrus were also observed at 50 mg/kg bw/day (both $P<0.05$), but to a lesser extent as seen at the highest dose level. The day at vaginal opening in the 100 mg/kg bw/day 4-nonylphenol treated animals was observed in the presence of reduced body weight at day of vaginal opening.

Table 9 Absolute and relative organ weights in Sprague-Dawley rats treated with nonylphenol or DES in the female pubertal onset assay as reported by Kim et al. 2002 (37).

Outcome parameter		Control	Nonylphenol 10 mg/kg	Nonylphenol 50 mg/kg	Nonylphenol 100 mg/kg	DES 0.2 µg/kg	DES 1.0 µg/kg	DES 5.0 µg/kg
Body weight (g)	**Initial [a]**	52.5 ± 3.6	50.5 ± 2.9	50.8 ± 2.5	50.7 ± 2.1	51.0 ± 3.2	53.6 ± 2.9	53.8 ±3.4
	Final [b]	156.7 ± 5.7	158.3 ± 7.1	153.2 ± 11.9	153.3 ± 10.2	168.9 ± 12.1	168.9 ± 6.5	159 ± 8.9
Thyroid glands	**Absolute (mg)**	8.91 ± 1.68	9.05 ± 2.11	9.24 ± 1.18	9.16 ± 2.43	9.01 ± 2.03	9.93 ± 3.17	11.27 ± 4.35*
	Relative (mg/100g bw)	5.66 ± 0.99	5.68 ± 1.47	5.80 ± 0.98	5.91 ± 1.27	6.01 ± 2.25	6.43 ± 2.54	7.01 ± 2.59*
Combined ovaries	**Absolute (mg)**	54.77 ± 9.11	58.42 ± 5.05	60.63 ± 6.08	45.58 ± 7.92*	56.30 ± 10.28	51.95 ± 6.59	40.04 ± 8.62*
	Relative (mg/100g bw)	34.86 ± 6.21	36.89 ± 3.18	38.23 ± 3.29	29.56 ± 3.87*	33.55 ± 7.02	30.74 ± 3.67	25.26 ± 6.33*
Uterus	**Absolute (g)**	0.28 ± 0.07	0.28 ± 0.08	0.28 ± 0.08	0.21 ± 0.03*	0.30 ± 0.05	0.34 ± 0.03	0.29 ± 0.06
	Relative (g/100g bw)	0.18 ± 0.05	0.18 ± 0.05	0.18 ± 0.04	0.13 ± 0.04*	0.18 ± 0.03	0.19 ± 0.02	0.18 ± 0.04

Mean ± S.D. (n = 10 animals per treatment group).
[a] Body weight on the first day of treatment (21 days of age).
[b] Body weight on the day of necropsy (41 days of age).
* Significantly different from control by Dunnett's test (P <0.05).

Table 10 Absolute organ weights in Sprague-Dawley rats treated with nonylphenol or DES in the female pubertal onset assay as reported by Kim et al. 2002 (37).

Outcome parameter		Control	Nonylphenol 10 mg/kg	Nonylphenol 50 mg/kg	Nonylphenol 100 mg/kg	DES 0.2 µg/kg	DES 1.0 µg/kg	DES 5.0 µg/kg
Body weight (g)	**Initial [a]**	52.5 ± 3.6	50.5 ± 2.9	50.8 ± 2.5	50.7 ± 2.1	51.0 ± 3.2	53.6 ± 2.9	53.8 ± 3.4
	Final [b]	156.7 ± 5.7	158.3 ± 7.1	153.2 ± 11.9	153.3 ± 10.2	168.9 ± 12.1	168.9 ± 6.5	159.4 ± 8.9
Liver (g)		5.71 ± 0.29	5.99 ± 0.54	5.80 ± 0.55	5.44 ± 0.61	6.58 ± 0.66	7.23 ± 1.32	6.05 ± 0.65
Heart (g)		0.65 ± 0.04	0.63 ± 0.04	0.65 ± 0.05	0.60 ± 0.05	0.68 ± 0.06	0.66 ± 0.08	0.64 ± 0.06
Kidneys (g)		1.49 ± 0.08	1.48 ± 0.13	1.47 ± 0.12	1.33 ± 0.16*	1.47 ± 0.14	1.51 ± 0.07	1.51 ± 0.14
Thymus (g)		0.48 ± 0.09	0.61 ± 0.06	0.56 ± 0.07	0.57 ± 0.05	0.61 ± 0.07	0.54 ± 0.09	0.50 ± 0.05
Pituitary (mg)		10.30 ± 1.68	8.63 ± 1.04	9.10 ± 1.65	7.74 ± 0.98*	9.12 ± 2.23	8.89 ± 3.31	9.54 ± 1.14
Adrenals (mg)		42.43 ± 6.32	35.57 ± 3.28	38.42 ± 5.25	38.64 ± 4.23	41.71 ± 6.08	43.20 ± 9.13	41.22 ± 3.54

Mean ± S.D. (n = 10 animals per treatment group).
[a] Body weight on the first day of treatment (21 days of age).
[b] Body weight on the day of necropsy (41 days of age).
* Significantly different from control by Dunnett's test (P <0.05).

Table 11 Effects of nonylphenol and DES on vaginal opening and serum hormone concentration in female pubertal onset assay as reported by Kim et al. 2002 (37).

Outcome parameter		Control	Nonylphenol 10 mg/kg	Nonylphenol 50 mg/kg	Nonylphenol 100 mg/kg	DES 0.2 µg/kg	DES 1.0 µg/kg	DES 5.0 µg/kg
Body weight (g)	**Initial [a]**	52.5 ± 3.6	50.5 ± 2.9	50.8 ± 2.5	50.7 ± 2.1	51.0 ± 3.2	53.6 ± 2.9	53.8 ± 3.4
	Final [b]	156.7 ± 5.7	158.3 ± 7.1	153.2 ± 11.9	153.3 ± 10.2	168.9 ± 12.1	168.9 ± 6.5	159.4 ± 8.9
VO [c] (day)		32.4 ± 1.6	33.0 ± 1.3	31.5 ± 1.4*	26.3 ± 1.5*	32.4 ± 1.4	29.7 ± 2.9*	24.1 ± 0.3*
Body weight at VO day (g)		117.4 ± 14.8	118.7 ± 12.2	108.7 ± 13.1	74.1 ± 9.4*	125.5 ± 14.3	103.3 ± 21.2	66.7 ± 4.7*
TSH (ng/mL)		0.61 ± 0.20	0.82 ± 0.29	0.86 ± 0.36	0.85 ± 0.25	0.78 ± 0.16	0.81 ± 0.27	0.96 ± 0.23*
T4 (g/dL)		3.24 ± 0.16	3.30 ± 0.13	3.15 ± 0.14	2.63 ± 0.15*	3.49 ± 0.11	3.12 ± 0.18	2.40 ± 0.01*

Mean ± S.D. (n = 10 animals per treatment group).
[a] Body weight on the first day of treatment (21 days of age).
[b] Body weight on the day of necropsy (41 days of age).
[c] VO: vaginal opening.
* Significantly different from control by Dunnett's test (P <0.05).

Table 12 Effects of nonylphenol and DES on oestrous cyclicity in prepubertal Sprague–Dawley rats during 20-day treatment in female pubertal onset assay as reported by Kim et al. 2002 (37).

Outcome parameter		**Control**	**Nonylphenol 10 mg/kg**	**Nonylphenol 50 mg/kg**	**Nonylphenol 100 mg/kg**	**DES 0.2 µg/kg**	**DES 1.0 µg/kg**	**DES 5.0 µg/kg**
Days of	**Proestrus**	1.40 ± 0.52	0.90 ± 0.74	1.20 ± 0.63	2.10 ± 0.88	0.90 ± 0.57	1.30 ± 0.48	0.81 ± 0.79
	Estrus	2.00 ± 0.82	1.00 ± 0.82	1.70 ± 0.82	2.44 ± 0.73	1.40 ± 0.70	2.80 ± 0.63	13.00 ± 2.49*
	Diestrus	5.30 ± 0.67	6.10 ± 1.10	6.60 ± 1.07*	10.90 ± 1.85*	4.00 ± 0.94	6.20 ± 1.40	3.50 ± 1.18*

Mean ± S.D. (n = 10 animals per treatment group).
* Significantly different from control by Dunnett's test (P <0.05).

Postnatal exposure study in rats (Willoughby et al., 2005)
The effects of 4-nonylphenol on onset of puberty and reproductive development was measured in 1-day old female Sprague-Dawley rats (38). The animals were exposed to 0, 5 or 50 mg/kg bw/day 4-nonylphenol on postnatal day 1-10 by subcutaneous injection. Further, additional groups were treated with 4-tert-octylphenol and DES. The day of vaginal opening, ovulation, prepubertal LH levels, LH response to oestradiol, oestrous cyclicity, and ovarian histology were determined. No significant effects were observed upon treatment with 4-nonylphenol on any of the observed parameters. Results were given in figures; no absolute number were presented.

8.1.2 *Human data*
Human data on 4-nonylphenol (CAS 104-40-5) describing adverse effects on fertility/sexual function were not found.
The available human data comprised of studies performed where the exposure to the type of nonylphenol was unclear or where study participants were exposed to multiple nonylphenols. Therefore, these studies were not included in this section.

8.2 Adverse effects on development

8.2.1 *Animal data*
Table 13 summarizes a single study of 4-nonylphenol in experimental animals focusing on adverse effects on development. The test substance concerns linear 4-nonylphenol only (CAS# 104-40-5).

Table 13 Summary table of an animal study on adverse effects on development

Reference	Species	Experimental period and design	Dose and route	General toxicity	Developmental toxicity	Remarks
Oral						
Couderc et al., 2014 (40)	Rat, Sprague Dawley, females. n=7/group; age unknown After delivery, the number of pups was adjusted to 3 males and 3 females per dam.	Neurodevelopmental and behavioural effects in F1 rats. Daily exposure from GD5 until PND21. Effect parameters: Reproduction success, macroscopic organ abnormalities, hepato-somatic index (HSI), thyroid-somatic index (TSI) and gonado-somatic index (GSI). Day of appearance of opening eyes, eruption of upper and lower incisors and auditory startle. (all pups) Limb grasping, crossed extensor reflex, gait, negative geotaxis. (n=8 per group)	Test item: 4-nonylphenol (Cas# 104-40-5); Route of exposure: oral gavage Exposure levels: 0, 50 and 200 mg/kg bw/day Negative control: vehicle (corn oil) No positive control	<u>Dams 200 mg/kg bw/day</u> - Decreased relative weight gain at GDs 5-7 and during the 3rd week of lactation. - Longer gestational duration. <u>F1, female, 200 mg/kg bw/day</u> - Decreased pup weight gain compared to control and 50 mg/kg group at PND2 and PND21 - TSI was decreased at PND 21 and 75 <u>F1, male, 200 mg/kg bw/day</u> - TSI was decreased at PND 21 <u>F1, female, 50 mg/kg bw/day</u> - Decreased weight gain after weaning	<u>Male and female, 200 mg/kg bw/day</u> Eye opening and upper incisors eruption occurred earlier. *Open field test* <u>Male, 200 mg/kg bw/day</u> Increased activity (multiple parameters) at PND36 <u>Female, 200 mg/kg bw/day</u> - Higher frequency of high mobility period at PND 72 <u>Male, 50 mg/kg bw/day</u> - Increase in high mobility duration and frequency *MWM test PND 29 and 33 (data on first 30 seconds of test)* Analyses of the whole time period of the MWM (90s) did not show effects of nonylphenol treatment <u>Female, 200 mg/kg bw/day</u>	No guideline study. No information on GLP.

Reference	Species	Experimental period and design	Dose and route	General toxicity	Developmental toxicity	Remarks
		Homing test, open field test, Morris Water Maze (MWM) test. (n=8 per group) Statistical analyses: - Factorial analyses of variance (ANOVA) or Kruskal-Wallis ANOVA was performed. Post hoc tests: least significant difference Fisher or multiple comparisons of p-values. - Cox-Mantel model (for homing test) - General linear model with repetition (for Morris Water Maze test)		- TSI was decreased at PND 75 <u>F1, male, 50 mg/kg bw/day</u> - Increased GSI compared to control and 200 mg/kg group at weaning <u>Gender differences</u> - Weight gain of males was higher from weaning to PND75 (all treatment groups) - TSI of females was higher and HSI of females was lower at PND 75 (control and 200 mg/kg group) - GSI of males was higher (all treatment groups and ages)	- Better short term memory (multiple parameters) - Increase in degree of heading to point <u>Female, 50 mg/kg bw/day</u> - Increase in degree of heading to point *MWM test PND 64 and 69 (data on first 30 seconds of test)* <u>Females 200 mg/ kg bw/day</u> -Reached the platform earlier than controls. - Spent more time in zone 4 and were closest to the platform zone. - Spent more time in the platform zone <u>Males, 200 mg/kg bw/day</u> - Lower meander value <u>Females, 50 mg/kg bw/day</u> - Spent more time in zone 4 and were closer to the platform zone - Moved less and slower than controls	

Neurodevelopmental and behavioural effects in rats (Couderc et al., 2014)

A one-generation study in rats on neurodevelopmental and behavioural effects was performed by Couderc et al. (40). Dams (n=7 per group) were treated with 4-n-nonylphenol at a dose of 50 or 200 mg/kg bw/day from GD 5 until PND 21 by oral gavage. The body weights of all animals were monitored. Hepato-somatic index (HSI), gonad-somatic index (GSI) and thyroid-somatic index (TSI) were calculated. Day of appearance of opening eyes, eruption of upper and lower incisors and auditory startle was noted. Neurological reflexes and motor coordination tests were carried out as well as a homing test and two Morris Water Maze (MWM) tests.

Signs of general toxicity were observed in dams (200 mg/kg bw/day) and included lower relative weight gain. Also increased gestational duration was noticed. Pup weight gain of females (200 mg/kg bw/day) was significantly decreased at PND2 and PND21 and in the 50 mg/kg bw/day group after weaning. A decreased TSI was observed in male and female pups at multiple dose groups and points in time. In male pups (50 mg/kg bw/day), GSI was higher than in controls and the high dose group at weaning ($P<0.05$).

Data on days of appearance of physical and sensorimotor signs are presented in table 14. In the 200 mg/kg bw/day group (M+F combined) eye opening and upper incisors eruption occurred earlier ($P<0.05$).

No significant difference in homing performance at PND 14 was observed (data not shown). Data on the open-field tests are shown in table 15 and table 16. Males (200 mg/kg bw/day) were more active compared to controls at PND36 ($P<0.05$ for various test parameters). At PND 71, females (200 mg/kg bw/day) presented a higher frequency of high mobility period compare to control ($P<0.05$). In males (50 mg/kg bw/day) an increase in high mobility duration and frequency was observed ($P<0.05$).

Results of the first and second MWM tests are presented in table 17 and table 18. Females (200 mg/kg bw/day) showed better short term memory (1st MWM test, $P<0.01$ for various test parameters) and both treatment groups showed an increase in degree of heading to point ($P<0.05$). In the second MWM test, no effects were observed on short term memory. Females (200 mg/kg bw/day) reached the platform earlier than controls ($P<0.05$). Males (200 mg/kg bw/day) had a lower meander value compared to controls ($P<0.05$). Females in the first (50 mg/kg bw/day and 200 mg/kg bw/day), spent more time in zone 4 and were closest to the platform zone ($P<0.05$). Females (200 mg/kg bw/day) spent more time in the platform zone compared to controls ($P<0.05$) and females (50 mg/kg bw/day), moved less and slower than controls ($P<0.05$).

Table 14 Effect of nonylphenol on day of appearance on pups of physical and sensorimotor signs according to Courderc et al. (40)

	Days of appearance		
	0 mg/kg/day	**50 mg/kg/day**	**100 mg/kg/day**
Physical maturation			
Upper incisors eruption	9.6 ± 0.4	9.3 ± 0.3	9.0 ± 0.5*
Lower incisors eruption	9.5 ± 0.4	9.5 ± 0.4	9.1 ± 0.7
Eye opening	15.6 ± 0.6	15.4 ± 0.4	14.9 ± 0.7*
Auditory startle	12.5 ± 0.4	12.2 ± 0.3	12.6 ± 0.5
Sensorimotor development			
Forelimb grasp	3.3 ± 0.4	3.2 ± 0.2	3.7 ± 0.8
Hindlimb grasp	6.3 ± 0.9	5.8 ± 0.7	6.5 ± 1.5
Crossed extensor reflex	14.6 ± 0.5	14.6 ± 0.4	14.2 ± 0.7
Negative geotaxis	5.5 ± 0.5	5.6 ± 0.7	6.0 ± 0.5
Negative geotaxis + ascent	12.9 ± 1.2	12.7 ± 0.9	13.2 ± 1.1
Gait	6.6 ± 0.6	6.5 ± 0.7	6.7 ± 0.8

Days of appearance values are expressed as mean ± standard deviation (n = 8 per treatment group). *p < 0.05, **p < 0.01 and ***p < 0.001 compared with control

Table 15 Open field tests data on PND 36 as reported in Courderc et al. (40)

	Male			**Female**		
PND 36	**0 mg/kg/day**	**50 mg/kg/day**	**200 mg/kg/day**	**0 mg/kg/day**	**50 mg/kg/day**	**200 mg/kg/day**
Total distance moved (cm)	4246 ± 629	4832 ± 582	5147 ± 586 **	5619 ± 684 [c]	5825 ± 625 [b]	5557 ± 946
Mean velocity (cm/s)	7.08 ± 1.05	8.06 ± 0.97	8.59 ± 0.98**	9.40 ± 1.14 [c]	9.70 ± 1.04 [b]	9.27 ± 1.58
Mean distance to wall (cm)	14.7 ± 2.2	14.9 ± 1.0	15.6 ± 1.1	15.4 ± 0.6	15.1 ± 1.3	15.0 ± 1.0
Visited zone numbers	29.9 ± 20.0	35.4 ± 15.2	43.8 ± 15.5	54.1 ± 12.5 [a]	49.5 ± 18.0	52.3 ± 19.5
In zone						
In border duration (s)	519 ± 79	512 ± 26	492 ± 42	500 ± 18 [a]	500 ± 40	511 ± 31
In border frequency	37.3 ± 20.8	42.6 ± 10	50.4 ± 14.8	54.5 ± 15.2	46.1 ± 15.6	47.0 ± 11.2
In centre duration (s)	81.5 ± 79.0	88.0 ± 26.5	107 ± 42.57	99.8 ± 18.1 [a]	100 ± 41	88.3 ± 31.2
In centre frequency (s)	37.0 ± 20.6	42.9 ± 11.0	50.3 ± 15.1	54.6 ± 15.4	46.0 ± 15.6	47.0 ± 11.2
Mobility						
Immobile duration (s)	466 ± 29	442 ± 27	417 ± 30**	408 ± 34 [b]	393 ± 26 [b]	398 ± 45
Immobile frequency	360 ± 63	437 ± 59*	466 ± 45**	438 ± 69 [a]	500 ± 56 [a]	460 ± 70
Mobile duration (s)	132 ± 30	156 ± 26	181 ± 29**	189 ± 31 [b]	204 ± 26 [b]	198 ± 43
Mobile frequency	365 ± 64	445 ± 66*	483 ± 53***	458 ± 86 [a]	519 ± 56 [a]	487 ± 84
High mobile duration (s)	0.93 ± 0.88	1.35 ± 1.12	2.27 ± 1.65*	3.00 ± 2.81	3.10 ± 1.54 [a]	4.21 ± 2.58
High mobile frequency	7.50 ± 5.18	12.9 ± 10.5	24.4 ± 18.2	25.5 ± 23.8	25.9 ± 10.3 [a]	38.0 ± 25.4
Movement						
Moving duration (s)	335 ± 40	393 ± 29**	402 ± 24***	400 ± 34 [b]	427 ± 23 [a]	400 ± 45
Not moving duration	265 ± 40	206 ± 30**	198 ± 24***	200 ± 34 [c]	173 ± 23 [a]	200 ± 45
Stress						
Number of faeces	2.25 ± 2.55	1.50 ± 1.93	2.25 ± 2.43	2.38 ± 2.67	1.50 ± 2.14	1.88 ± 2.75
Rearing/leaning	49.1 ± 16.7	63.4 ± 17.0	69.6 ± 13.3*	72.9 ± 16.9 [a]	70.3 ± 16.9	72.1 ± 20.8
Grooming	3.63 ± 1.60	2.75 ± 1.39	4.25 ± 2.05	3.25 ± 1.67	4.00 ± 3.51	3.50 ± 1.41

Values are mean ± SD; n = 8. *p < 0.05, **p < 0.01, ***p < 0.001 significantly compared with control. Letter indicated gender differences by treatment groups (control, 50 and 200 mg/kg/day): [a] p< 0.05, [b] p< 0.01 and [c] p< 0.001.

Table 16 Open field tests data on PND 71 as reported in Courderc et al. (40)

	Male			**Female**		
PND 71	**0 mg/kg/day**	**50 mg/kg/day**	**200 mg/kg/day**	**0 mg/kg/day**	**50 mg/kg/day**	**200 mg/kg/day**
Total distance moved(cm)	4035 ± 927	4283 ± 859	4523 ± 890	5962 ± 1013 [b]	5931 ± 713 [c]	5875 ± 586 [b]
Mean velocity (cm/s)	6.73 ± 1.55	7.14 ± 1.43	7.54 ± 1.48	9.94 ± 1.69 [b]	9.89 ± 1.19 [c]	9.80 ± 0.97 [b]
Mean distance to wall (cm)	9.02 ± 2.22	9.73 ± 1.31	8.98 ± 1.06	9.60 ± 0.78	9.48 ± 0.91	9.28 ± 0.64
Visited zone numbers	27.5 ± 18.0	34.1 ± 18.8	31.1 ± 19.2	58.9 ± 11.5 [c]	66.5 ± 14.3 [b]	65.0 ± 18.1 [b]
In zone						
In border duration (s)	139 ± 95	186 ± 64	144 ± 52	172 ± 39	174 ± 37	166 ± 31
In border frequency	44.6 ± 17.8	58.6 ± 18.8	59.6 ± 14.4	70.5 ± 12.3 [b]	59.5 ± 11.1	58.6 ± 4.2
In centre duration (s)	461 ± 95	414 ± 64	456 ± 51	428 ± 39	426 ± 37	434 ± 31
In centre frequency (s)	44.6 ± 17.5	59.4 ± 18.7	59.9 ± 14.6	71.3 ± 12.5 [b]	60.0 ± 11.1	59.1 ± 4.1
Mobility						
Immobile duration (s)	409 ± 38	404 ± 28	393 ± 25	363 ± 29 [a]	358 ± 15 [b]	358 ± 15 [b]
Immobile frequency	1184 ± 145	1235 ± 95	1243 ± 71	1206 ± 83	1238 ± 68	1250 ± 40
Mobile duration (s)	190 ± 38	193 ± 27	204 ± 24	228 ± 28a	232 ± 14 [b]	230 ± 15 [b]
Mobile frequency	1180 ± 144	1224 ± 93	1234 ± 68	1204 ± 71	1224 ± 70	1240 ± 35
High mobile duration (s)	1.15 ± 0.74	3.53 ± 3.24*	3.22 ± 3.20	8.87 ± 2.90 [c]	10.2 ± 3.3 [b]	12.2 ± 4.3 [c]
High mobile frequency	9.25 ± 4.98	31.8 ± 27.2*	31.9 ± 30.4	80.8 ± 24.5 [c]	96.9 ± 27.3 [b]	114 ± 38* [c]
Movement						
Moving duration (s)	191 ± 39	196 ± 28	207 ± 25	237 ± 29 [a]	242 ± 15 [b]	242 ± 16 [b]
Not moving duration	409 ± 38	404 ± 28	393 ± 25	363 ± 29 [a]	358 ± 15 [b]	358 ± 16 [b]
Stress						
Number of faeces	1.13 ± 2.10	0.88 ± 2.47	0.50 ± 1.07	0.50 ± 1.41	0.00 ± 0.00	0.00 ± 0.00
Rearing/leaning	57.1 ± 12.6	67.4 ± 20.0	73.8 ± 22.2	93.0 ± 14.7 [c]	91.4 ± 15.0 [a]	98.8 ± 14.9 [a]
Grooming	3.88 ± 2.47	3.50 ± 2.20	5.75 ± 2.19	2.88 ± 1.46	3.38 ± 1.69	4.50 ± 3.66

Values are mean ± SD; n = 8. *p < 0.05, **p < 0.01, ***p < 0.001 significantly compared with control. Letter indicated gender differences by treatment groups (control, 50 and 200 mg/kg/day): [a] p< 0.05, [b] p< 0.01 and [c] p< 0.001.

Table 17 Morris Water Maze data on short term (A) and long term memory (B) on PNDs 26-34 as provided in Courderc et al. (40)

	Male			**Female**		
A	**0 mg/kg/day**	**50 mg/kg/day**	**200 mg/kg/day**	**0 mg/kg/day**	**50 mg/kg/day**	**200 mg/kg/day**
Total distance moved(cm)	925 ± 151	917 ± 158	858 ± 115	941 ± 97	917 ± 131	917 ± 108
Total distance to zone platform (cm)	6222 ± 2657	6618 ± 1220	5975 ± 2097	7888 ± 1948 [b]	6262 ± 885	5404 ± 683**
Number of visited zones	10.8 ± 3.0	11.5 ± 2.4	9.63 ± 1.60	11.3 ± 1.6	10.1 ± 3.0	9.88 ± 4.02
Velocity mean (cm/s)	31.1 ± 5.1	30.8 ± 5.3	28.8 ± 3.9	31.6 ± 3.3	30.8 ± 4.4	30.8 ± 3.6
In zone						
In zone 4 duration (s)	14.0 ± 5.8	13.4 ± 3.0	15.2 ± 3.3	10.5 ± 4.2 [a]	13.1 ± 2.6	16.3 ± 2.8**
In zone 4 latency to first (s)	3.73 ± 5.62	3.25 ± 2.54	3.70 ± 2.34	6.40 ± 6.50	2.53 ± 2.19	3.83 ± 3.50
In zone platform duration (s)	3.17 ± 1.12	1.93 ± 0.94	2.78 ± 1.88	1.83 ± 1.07 [a]	2.13 ± 0.72	2.80 ± 0.78*
In centre frequency (s)	4.43 ± 2.86	4.95 ± 4.53	4.85 ± 2.52	9.75 ± 9.76	3.68 ± 2.33	5.73 ± 4.33
Mean meander (deg/cm)	7.20 ± 5.41	8.27 ± 5.80	10.6 ± 8.3	11.8 ± 13.7	7.22 ± 3.60	9.77 ± 10.66
Mean heading to point (deg)	110 ± 32	103 ± 20	93.4 ± 27.4	99.3 ± 30.8	100 ± 14	95.2 ± 20.6
B						
Total distance moved(cm)	932 ± 184	963 ± 69	913 ± 143	923 ± 91	997 ± 108	942 ± 148
Total distance to zone platform (cm)	7076 ± 2103	8253 ± 1828	7730 ± 1729	8456 ± 1625	6890 ± 1567	7708 ± 1962
Number of visited zones	11.0 ± 3.7	11.9 ± 2.4	10.5 ± 1.3	9.88 ± 1.25	12.3 ± 2.7*	10.6 ± 2.6
Velocity mean (cm/s)	31.3 ± 6.2	32.3 ± 2.3	30.7 ± 4.8	31.0 ± 3.1	33.5 ± 3.6	31.7 ± 5.0
In zone						
In zone 4 duration (s)	12.0 ± 3.7	10.6 ± 3.6	11.7 ± 3.8	10.2 ± 4.3	12.5 ± 3.4	9.63 ± 3.51
In zone 4 latency to first (s)	2.90 ± 2.08	3.45 ± 2.66	3.95 ± 2.31	4.78 ± 2.54	3.18 ± 1.51	6.68 ± 7.22
In zone platform duration (s)	2.15 ± 0.98	1.70 ± 0.34	2.25 ± 1.27	1.77 ± 0.72	2.10 ± 1.13	1.63 ± 0.99
In centre frequency (s)	4.53 ± 2.93	4.25 ± 2.82	4.90 ± 2.75	5.60 ± 2.42	4.00 ± 1.80	7.90 ± 7.86
Mean meander (deg/cm)	10.6 ± 11.8	11.30 ± 7.20	8.33 ± 5.55	11.1 ± 6.9	7.08 ± 4.80	6.88 ± 5.48
Mean heading to point (deg)	100 ± 12	86.9 ± 19.2	92.0 ± 29.0	66.9 ± 31.8 [a]	97.1 ± 22.6*	93.6 ± 14.0*

The data were obtained during the first 30s of these tests. The MWM STM was realised at PND 29 and the MWM LTM at PND 33. Values are mean ± SD; n = 8. *p<0.05, **p<0.01 and ***p <0.001 significantly compared with control. Letter indicated gender differences by treatment groups (control, 50 and 200 mg/kg/day): [a]p<0.05, [b]p<0.01 and [c] p< 0.001.

Table 18 Morris Water Maze data on short term (A) and long term memory (B) on PNDs 61-69 as provided in Courderc et al. (40)

	Male			**Female**		
A	**0 mg/kg/day**	**50 mg/kg/day**	**200 mg/kg/day**	**0 mg/kg/day**	**50 mg/kg/day**	**200 mg/kg/day**
Total distance moved(cm)	815 ± 135	797 ± 95	750 ± 92	948 ± 106 [a]	864 ± 133	907 ± 145 [a]
Total distance to zone platform (cm)	5784 ± 1424	6747 ± 940	6402 ± 1118	7052 ± 1575	7506 ± 773	6681 ± 879
Number of visited zones	12.1 ± 3.0	9.38 ± 2.13	10.1 ± 3.8	12.0 ± 3.7	11.3 ± 3.0	12.4 ± 2.5
Velocity mean (cm/s)	27.3 ± 4.5	26.8 ± 3.2	25.2 ± 3.0	31.8 ± 3.6 [a]	29.01 ± 4.47	30.4 ± 4.9a
In zone						
In zone 4 duration (s)	13.3 ± 3.7	11.6 ± 2.7	13.1 ± 2.5	10.7 ± 3.7	10.3 ± 2.3	11.7 ± 2.7
In zone 4 latency to first (s)	2.40 ± 1.80	3.03 ± 1.58	3.95 ± 2.04	2.65 ± 1.12	3.35 ± 1.59	2.53 ± 1.00
In zone platform duration (s)	2.88 ± 1.65	1.93 ± 0.96	2.45 ± 1.32	1.93 ± 1.12	2.00 ± 0.76	2.58 ± 1.32
In centre frequency (s)	4.10 ± 3.57	3.95 ± 1.70	5.03 ± 2.17	4.55 ± 1.63	4.20 ± 1.99	4.08 ± 2.87
Mean meander (deg/cm)	12.7 ± 14.7	8.53 ± 3.70	7.23 ± 4.34	8.63 ± 5.48	10.7 ± 5.0	6.60 ± 4.33
Mean heading to point (deg)	100.8 ± 38.7	94.5 ± 50.3	105 ± 31	78.0 ± 33.6	87.8 ± 50.1	101 ± 36
B						
Total distance moved(cm)	798 ± 152	781 ± 100	833.88 ± 149.45	914 ± 100	802 ± 57*	879 ± 109
Total distance to zone platform (cm)	7662 ± 1463	7846 ± 1437	7090 ± 1241	8801 ± 1254	7469 ± 946*	7197 ± 1052**
Number of visited zones	9.88 ± 2.90	9.75 ± 1.04	10.3 ± 1.8	11.3 ± 2.6	9.25 ± 3.24	12.1 ± 2.2
Velocity mean (cm/s)	26.8 ± 5.1	26.21 ± 3.34	28.0 ± 5.0	30.7 ± 3.4	26.9 ± 1.9*	29.49 ± 3.67
In zone						
In zone 4 duration (s)	8.05 ± 4.40	9.10 ± 3.38	10.8 ± 3.4	7.03 ± 2.54	10.5 ± 2.8*	10.6 ± 2.8*
In zone 4 latency to first (s)	4.03 ± 3.09	4.45 ± 3.82	3.35 ± 2.95	4.70 ± 3.55	3.78 ± 3.51	2.55 ± 1.19
In zone platform duration (s)	1.13 ± 0.76	1.45 ± 0.83	1.80 ± 0.92	0.95 ± 0.75	2.03 ± 1.21	2.25 ± 1.50*
In centre frequency (s)	8.08 ± 9.15	5.20 ± 3.78	4.08 ± 3.04	5.78 ± 7.70	4.80 ± 3.28	3.20 ± 1.06
Mean meander (deg/cm)	14.0 ± 5.5	10.4 ± 4.9	8.06 ± 4.18*	9.53 ± 5.15	14.29 ± 8.64	8.23 ± 6.01
Mean heading to point (deg)	91.5 ± 25.7	100 ± 25	81.5 ± 29.8	96.7 ± 19.8	92.0 ± 10.9	95.2 ± 6.1

The data were obtained during the first 30s of these tests. The MWM STM was realised at PND 29 and the MWM LTM at PND 33. Values are mean ± SD; n = 8. *p<0.05, **p<0.01 and ***p <0.001 significantly compared with control. Letter indicated gender differences by treatment groups (control, 50 and 200 mg/kg/day): [a]p<0.05, [b]p<0.01 and [c] p< 0.001.

8.2.2 *Human data*

Human data on 4-nonylphenol (CAS 104-40-5) describing adverse effects on development were not found.
The available human data comprised of studies performed where the exposure to the type of nonylphenol was unclear or where study participants were exposed to multiple nonylphenols. Therefore, these studies were not included in this section.

8.3 Adverse effects on or via lactation

There were no animal or human studies available describing adverse effects on or via lactation, that were specific for nonylphenol with CAS number 104-40-5.

9 References

1. European Comission. Regulation (EC) No 1272/2008 of the European Parliament on classification, labelling and packaging of substances and mixtures [Available from: http://data.europa.eu/eli/reg/2008/1272/oj
2. EU-RAR. 4-nonylphenol (branched) and nonylphenol. Cas no. 84852-15-3 and 25154-52-3. Risk assessment. 1998.
3. Bontje D, Hermens, J., Vermeire, T., Damstra, . Integrated risk assessment: nonylphenol case study. WHO/IPCS/IRA. 2002.
4. Soares A, Guieysse B, Jefferson B, Cartmell E, Lester JN. Nonylphenol in the environment: a critical review on occurrence, fate, toxicity and treatment in wastewaters. Environ Int. 2008;34(7):1033-49.
5. Niu Y, Zhang J, Duan H, Wu Y, Shao B. Bisphenol A and nonylphenol in foodstuffs: Chinese dietary exposure from the 2007 total diet study and infant health risk from formulas. Food Chem. 2015;167:320-5.
6. Rudel RA, Camann DE, Spengler JD, Korn LR, Brody JG. Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. Environmental science & technology. 2003;37(20):4543-53.
7. Inoue K, Yoshida S, Nakayama S, Ito R, Okanouchi N, Nakazawa H. Development of stable isotope dilution quantification liquid chromatography-mass spectrometry method for estimation of exposure levels of bisphenol A, 4-tert-octylphenol, 4-nonylphenol, tetrabromobisphenol A, and pentachlorophenol in indoor air. Archives of environmental contamination and toxicology. 2006;51(4):503-8.
8. Moeder M, Martin C, Harynuk J, Gorecki T, Vinken R, Corvini PF. Identification of isomeric 4-nonylphenol structures by gas chromatography-tandem mass spectrometry combined with cluster analysis. J Chromatogr A. 2006;1102(1-2):245-55.
9. Kim YS, Katase T, Sekine S, Inoue T, Makino M, Uchiyama T, et al. Variation in estrogenic activity among fractions of a commercial nonylphenol by high performance liquid chromatography. Chemosphere. 2004;54(8):1127-34.
10. Shekhar S, Sood S, Showkat S, Lite C, Chandrasekhar A, Vairamani M, et al. Detection of phenolic endocrine disrupting chemicals (EDCs) from maternal blood plasma and amniotic fluid in Indian population. General and comparative endocrinology. 2017;241:100-7.
11. Gyllenhammar I, Glynn A, Darnerud PO, Lignell S, van Delft R, Aune M. 4-Nonylphenol and bisphenol A in Swedish food and exposure in Swedish nursing women. Environ Int. 2012;43:21-8.
12. Kim K, Park H, Lee JH. Urinary concentrations of trichlorophenols in the Korean adult population: results of the National Human Biomonitoring Survey 2009. Environmental science and pollution research international. 2014;21(4):2479-85.
13. Park H, Kim K. Urinary Levels of 4-Nonylphenol and 4-t-Octylphenol in a Representative Sample of the Korean Adult Population. Int J Environ Res Public Health. 2017;14(8).
14. Thiele B, Heinke V, Kleist E, Guenther K. Contribution to the structural elucidation of 10 isomers of technical p-nonylphenol. Environmental science & technology. 2004;38(12):3405-11.

15. Russ AS, Vinken R, Schuphan I, Schmidt B. Synthesis of branched para-nonylphenol isomers: occurrence and quantification in two commercial mixtures. Chemosphere. 2005;60(11):1624-35.
16. Shao B, Hu J, Yang M. Nonylphenol ethoxylates and their biodegradation intermediates in water and sludge of a sewage treatment plant. Bulletin of environmental contamination and toxicology. 2003;70(3):527-32.
17. RAC., SEAC. Background document to the Opinion on the Annex XV dossier proposing restrictions on NONYLPHENOL ETHOXYLATE. ECHA/RAC/ RES-O-0000005317-74-01/F. 2014.
18. Müller S, Schmid P, Schlatter C. Pharmacokinetic behavior of 4-nonylphenol in humans. Environmental Toxicology and Pharmacology. 1998;5(4):257-65.
19. Monteiro-Riviere NA, Van Miller JP, Simon G, Joiner RL, Brooks JD, Riviere JE. Comparative in vitro percutaneous absorption of nonylphenol and nonylphenol ethoxylates (NPE-4 and NPE-9) through human, porcine and rat skin. Toxicology and industrial health. 2000;16(2):49-57.
20. Lin L, Zheng LX, Gu YP, Wang JY, Zhang YH, Song WM. Levels of environmental endocrine disruptors in umbilical cord blood and maternal blood of low-birth-weight infants. Zhonghua yu fang yi xue za zhi [Chinese journal of preventive medicine]. 2008;42(3):177-80.
21. Balakrishnan B, Thorstensen E, Ponnampalam A, Mitchell MD. Passage of 4-nonylphenol across the human placenta. Placenta. 2011;32(10):788-92.
22. Chen M, Fan Z, Zhao F, Gao F, Mu D, Zhou Y, et al. Occurrence and Maternal Transfer of Chlorinated Bisphenol A and Nonylphenol in Pregnant Women and Their Matching Embryos. Environmental Science and Technology. 2016;50(2):970-7.
23. Green T, Swain C, Van Miller JP, Joiner RL. Absorption, bioavailability, and metabolism of para-nonylphenol in the rat. Regulatory Toxicology and Pharmacology. 2003;38(1):43-51.
24. Knaak JB, Eldridge JM, Sullivan LJ. Excretion of certain polyethylene glycol ether adducts of nonylphenol by the rat. Toxicology and applied pharmacology. 1966;9(2):331-40.
25. Xiao Q, Li Y, Ouyang H, Xu P, Wu D. High-performance liquid chromatographic analysis of bisphenol A and 4-nonylphenol in serum, liver and testis tissues after oral administration to rats and its application to toxicokinetic study. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences. 2006;830(2):322-9.
26. Lee PC, Marquardt M, Lech JJ. Metabolism of nonylphenol by rat and human microsomes. Toxicology Letters. 1998;99(2):117-26.
27. Zalko D, Costagliola R, Dorio C, Rathahao E, Craved JP. In vivo metabolic fate of the xeno-estrogen 4-n-nonylphenol in Wistar rats. Drug Metabolism and Disposition. 2003;31(2):168-78.
28. Daidoji T, Inoue H, Kato S, Yokota H. Glucuronidation and excretion of nonylphenol in perfused rat liver. Drug metabolism and disposition: the biological fate of chemicals. 2003;31(8):993-8.
29. Daidoji T, Ozawa M, Sakamoto H, Sako T, Inoue H, Kurihara R, et al. Slow elimination of nonylphenol from rat intestine. Drug metabolism and disposition: the biological fate of chemicals. 2006;34(1):184-90.
30. Jeong SH, Jang JH, Cho HY, Lee YB. Gender differences in pharmacokinetics and tissue distribution of 4-n-nonylphenol in rats. Archives of Toxicology. 2019;93(11):3121-39.

31. Fu H, Di Q, Wang J, Jiang Q, Xu Q. Toxicokinetics and distribution in female rats after chronic nonylphenol exposure. Toxicology and industrial health. 2020;36(11):925-35.
32. Fennell TRM, J.P. Disposition and metabolism of p-nonylphenol in male and female rats. SOT conference abstract. 1997.
33. Liu T, Cao W, Di Q, Zhao M, Xu Q. Evaluation of toxicokinetics of nonylphenol in the adult female Sprague-Dawley rats using a physiologically based toxicokinetic model. Regulatory toxicology and pharmacology : RTP. 2019;105:42-50.
34. Doerge DR, Twaddle NC, Churchwell MI, Chang HC, Newbold RR, Delclos KB. Mass spectrometric determination of p-nonylphenol metabolism and disposition following oral administration to Sprague-Dawley rats. Reproductive toxicology (Elmsford, NY). 2002;16(1):45-56.
35. Hossaini A MD, A M Vinggaard, H Frandsen, J J Larsen In Utero Reproductive Study in Rats Exposed to Nonylphenol. Reprod Toxicol 2001;15(5):537-43.
36. Dalgaard M, Pilegaard K, Ladefoged O. In utero exposure to diethylstilboestrol or 4-n-nonylphenol in rats: Number of Sertoli cells, diameter and length of seminiferous tubules estimated by stereological methods. Pharmacology and Toxicology. 2002;90(2):59-65.
37. Kim HS, Shin JH, Moon HJ, Kang IH, Kim TS, Kim IY, et al. Comparative estrogenic effects of p-nonylphenol by 3-day uterotrophic assay and female pubertal onset assay. Reproductive toxicology (Elmsford, NY). 2002;16(3):259-68.
38. Willoughby KN, Sarkar AJ, Boyadjieva NI, Sarkar DK. Neonatally administered tert-octylphenol affects onset of puberty and reproductive development in female rats. Endocrine. 2005;26(2):161-8.
39. Kim HS, Shin JH, Moon HJ, Kang IH, Kim TS, Kim IY, et al. Comparative estrogenic effects of p-nonylphenol by 3-day uterotrophic assay and female pubertal onset assay. Reproductive Toxicology. 2002;16(3):259-68.
40. Couderc M, Gandar A, Kamari A, Allain Y, Zalouk-Vergnoux A, Herrenknecht C, et al. Neurodevelopmental and behavioral effects of nonylphenol exposure during gestational and breastfeeding period on F1 rats. NeuroToxicology. 2014;44:237-49.

10 Annexes

10.1 Literature concerning branched 4-nonylphenol (CAS #84852-15-3)

Animal or *in vitro* studies:

1. Altstoffe IU. Teratogenicity study in Wistar rats treated orally with "nonylphenol". IBR project no. 20-04-0502/00-91. 1992.[16]
2. Gaido KW, Leonard LS, Lovell S, Gould JC, Babai D, Portier CJ, et al. Evaluation of chemicals with endocrine modulating activity in a yeast-based steroid hormone receptor gene transcription assay. Toxicol Appl Pharmacol. 1997;143(1):205-12.
3. NTP. Final Report on the reproductive toxicity of nonylphenol (CAS #84852-15-3) administered in diet to Sprague-Dawley rats. 1997.
4. Cunny HC, Mayes BA, Rosica KA, Trutter JA, Van Miller JP. Subchronic toxicity (90-day) study with para-nonylphenol in rats. Regulatory toxicology and pharmacology : RTP. 1997;26(2):172-8.
5. Chapin RE, Delaney J, Wang Y, Lanning L, Davis B, Collins B, et al. The effects of 4-nonylphenol in rats: a multigeneration reproduction study. Toxicological sciences : an official journal of the Society of Toxicology. 1999;52(1):80-91.
6. De Jager C, Bornman MS, Van Der Horst G. I. The effect of p-nonylphenol, an environmental toxic ant with oestrogenic properties, on fertility potential in adult male rats. Andrologia. 1999;31(2):99-106.[17]
7. De Jager C, Bornman MS, Oosthuizen MC. II. The effect of p-nonylphenol on the fertility potential of male rats after gestational, lactational and direct exposure. Andrologia. 1999;31(2):107-13. [17]
8. Odum J, Pyrah IT, Soames AR, Foster JR, Van Miller JP, Joiner RL, et al. Effects of p-nonylphenol (NP) and diethylstilboestrol (DES) on the Alderley Park (Alpk) rat: comparison of mammary gland and uterus sensitivity following oral gavage or implanted mini-pumps. Journal of applied toxicology : JAT. 1999;19(5):367-78.[18]
9. Odum J, Pyrah ITG, Foster JR, Van Miller JP, Joiner RL, Ashby J. Comparative activities of p-nonylphenol and diethylstilbestrol in noble rat mammary gland and uterotrophic assays. Regulatory Toxicology and Pharmacology. 1999;29(2 I):184-95.[18]
10. Odum J, Ashby J. Neonatal exposure of male rats to nonylphenol has no effect on the reproductive tract. Toxicological sciences : an official journal of the Society of Toxicology. 2000;56(2):400-4.[18]
11. Nagao T, Wada K, Marumo H, Yoshimura S, Ono H. Reproductive effects of nonylphenol in rats after gavage administration: a two-

[16] As cited in RAR (2002). No report available. Also presented in REACH registration dossier of CAS 84852-15-3 as Unnamed Report (1992).

[17] In the studies of De Jager et al., the CAS-number was not mentioned but the reported catalogue number of the supplier nowadays corresponds to the CAS-number of branched-nonylphenol.

[18] In the studies of Odum et al., the CAS-number was not mentioned but the authors report that 4-nonylphenol was from the same batch as tested in Chapin et al. 1999.

generation study. Reproductive toxicology (Elmsford, NY). 2001;15(3):293-315.
12. Tyl RW, Myers CB, Marr MC, Castillo NP, Seely JC, Sloan CS, et al. Three-generation evaluation of dietary para-nonylphenol in CD (Sprague-Dawley) rats. Toxicological Sciences. 2006;92(1):295-310.
13. Arcanjo R, Rocha M, Marques M, Almeida-Santos MF, Báo SN. Effects of 4-Nonylphenol on reproduction of exposed females during puberty. Animal Reproduction. 2016;13(4):795-805.
14. Duan P, Hu C, Butler HJ, Quan C, Chen W, Huang W, et al. Effects of 4-nonylphenol on spermatogenesis and induction of testicular apoptosis through oxidative stress-related pathways. Reproductive Toxicology. 2016;62:27-38.
15. Cha S, Baek JW, Ji HJ, Choi JH, Kim C, Lee MY, et al. Disturbing Effects of Chronic Low-dose 4-Nonylphenol exposing on Gonadal Weight and Reproductive Outcome over One-generation. Development & reproduction. 2017;21(2):121-30.
16. Kusum K, Ashish A, Singh R, Chaube R. Exposure of 4-nonylphenol: Effect on physiology and reproductivity of male mice. Journal of Clinical and Diagnostic Research. 2021;15(3):AC01-AC5.

Human studies

17. Chen M, Fan Z, Zhao F, Gao F, Mu D, Zhou Y, et al. Occurrence and Maternal Transfer of Chlorinated Bisphenol A and Nonylphenol in Pregnant Women and Their Matching Embryos. Environmental Science and Technology. 2016;50(2):970-7.

10.2 Literature concerning 4-nonylphenol with unspecified CAS-number

Animal or *in vitro* studies:

1. Hüls AG. Nonylphenol: 28 day oral (dietary) sub-acute toxicity study in the rat. Hazleton UK report no. 5917-671/1. 1989. As cited in RAR (2002). No report available.
2. Soto AM, Justicia H, Wray JW, Sonnenschein C. p-Nonyl-phenol: an estrogenic xenobiotic released from "modified" polystyrene. Environmental health perspectives. 1991;92:167-73.[19]
3. White R, Jobling S, Hoare SA, Sumpter JP, Parker MG. Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology. 1994;135(1):175-82.
4. ICI-Surfactants. Screening chemicals for effects on uterine growth in immature female rats: nonylphenol,octylphenol, and nonylphenoxyacetic acid. Zeneca CTL report no. CTL/R/1249. 1996. As cited in RAR (2002). Report not available.
5. Routledge EJ, Sumpter JP. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environmental Toxicology and Chemistry. 1996;15(3):241-8.

[19] In the study of Soto et al. the tested 4-nonylphenol was reported to consist of 85% branched side-chain isomers.

6. Hüls AG. Nonylphenol: 28 day oral (dietary) sub-acute toxicity study in the rat. Hazleton UK report no. 5917-671/1. 1989. As cited in RAR (2002) Report not available.
7. Soto AM, Justicia H, Wray JW, Sonnenschein C. p-Nonyl-phenol: an estrogenic xenobiotic released from "modified" polystyrene. Environmental health perspectives. 1991;92:167-73.[20]
8. White R, Jobling S, Hoare SA, Sumpter JP, Parker MG. Environmentally persistent alkylphenolic compounds are estrogenic. Endocrinology. 1994;135(1):175-82.
9. ICI-Surfactants. Screening chemicals for effects on uterine growth in immature female rats: nonylphenol,octylphenol, and nonylphenoxyacetic acid. Zeneca CTL report no. CTL/R/1249. 1996. As cited in RAR (2002). Report not available.
10. Routledge EJ, Sumpter JP. Estrogenic activity of surfactants and some of their degradation products assessed using a recombinant yeast screen. Environmental Toxicology and Chemistry. 1996;15(3):241-8.
11. Routledge EJ, Sumpter JP. Structural features of alkylphenolic chemicals associated with estrogenic activity. J Biol Chem. 1997;272(6):3280-8.
12. Odum J, Lefevre PA, Tittensor S, Paton D, Routledge EJ, Beresford NA, et al. The rodent uterotrophic assay: critical protocol features, studies with nonyl phenols, and comparison with a yeast estrogenicity assay. Regulatory toxicology and pharmacology : RTP. 1997;25(2):176-88.
13. Blom A, Ekman E, Johannisson A, Norrgren L, Pesonen M. Effects of xenoestrogenic environmental pollutants on the proliferation of a human breast cancer cell line (MCF-7). Archives of environmental contamination and toxicology. 1998;34(3):306-10.
14. Chemicals manufacturers association. Uterine weight assay of p-nonylphenol and p-octylphenol ethoxylate-5 (OPE-5) administered orally to ovariectomized Sprague Dawley rats. MB Research Labs project no. MB 96- 4960.07. 1997. As cited in RAR (2002). No report available.
15. Ferguson SA, Flynn KM, Delclos KB, Newbold RR. Maternal and offspring toxicity but few sexually dimorphic behavioral alterations result from nonylphenol exposure. Neurotoxicology and Teratology. 2000;22(4):583-91.
16. Nagao T. Disruption of the reproductive system and reproductive performance by administration of nonylphenol to newborn rats. Human and Experimental Toxicology. 2000;19(5):284-96.
17. De Jager C, Bornman MS, Wandrag S, Sharp VW. Lethal dose and reproductive parameters of p-Nonylphenol in rats. Archives of Andrology. 2001;46(3):183-7.
18. Fan Q, Li W, Shen L. Adverse effects of exposure to p-nonylphenol on reproductive system of young male rats. Zhonghua yu fang yi xue za zhi [Chinese journal of preventive medicine]. 2001;35(5):344-6. Article in Chinese, abstract only.

[20] In the study of Soto et al., the tested 4-nonylphenol was reported to consist of 85% branched side-chain isomers.

19. Laurenzana EM, Weis CC, Bryant CW, Newbold R, Delclos KB. Effect of dietary administration of genistein, nonylphenol or ethinyl estradiol on hepatic testosterone metabolism, cytochrome P-450 enzymes, and estrogen receptor alpha expression. Food and Chemical Toxicology. 2002;40(1):53-63.[21]
20. Laurenzana EM, Balasubramanian G, Weis C, Blaydes B, Newbold RR, Delclos KB. Effect of nonylphenol on serum testosterone levels and testicular steroidogenic enzyme activity in neonatal, pubertal, and adult rats. Chemico-Biological Interactions. 2002;139(1):23-41.[21]
21. Takagi H, Shibutani M, Masutomi N, Uneyama C, Mitsumori K, Hirose M. Effects of perinatal exposure of five putative endocrine disrupting chemicals (EDCs), methoxychlor, genistein, diisononylphthalate, 4-nonylphenol and bisphenol A, on endocrine/reproductive systems in rats. Abstract; (MEETING ABSTRACT). 2003. Article not available, abstract only.
22. Kyselova V, Peknicova J, Buckiova D, Boubelik M. Effects of p-nonylphenol and resveratrol on body and organ weight and in vivo fertility of outbred CD-1 mice. Reproductive Biology and Endocrinology. 2003;1.
23. Shimizu M, Yamano T, Noda T. Effects of prenatal and/or neonatal exposure to 4-nonylphenol or bisphenol A on the reproductive organs in male and female rats - Histological findings. Annual Report of Osaka City Institute of Public Health and Environmental Sciences. 2003;65:17-23. Article in Japanese, abstract only.
24. Zalko D, Costagliola R, Dorio C, Rathahao E, Craved JP. In vivo metabolic fate of the xeno-estrogen 4-n-nonylphenol in Wistar rats. Drug Metabolism and Disposition. 2003;31(2):168-78.
25. Long DX, Li Y, Pei XR. [Study on developmental toxicity of 4 - nonylphenol on cultured rat embryos]. Chung-Kuo Kung Kung Wei Sheng (China Public Health). 2004;20(1):13-5. Article in Chinese, abstract only.
26. Han XD, Tu ZG, Gong Y, Shen SN, Wang XY, Kang LN, et al. The toxic effects of nonylphenol on the reproductive system of male rats. Reproductive toxicology (Elmsford, NY). 2004;19(2):215-21.
27. Hunag YN, Cheng WB, Xu PY, Yu WS, Li QY, Wang WD, et al. Exploring the Sensitive Indicators of Uterotrophic Assay in Immature SD Rats Exposed to Para-nonylphenol. Journal of Sichuan University (Medical Science Edition). 2004;35(1):87-90. Article in Chinese, abstract only.
28. Moon HJ, Han SY, Shin JH, Kang IH, Kim TS, Hong JH, et al. Gestational exposure to nonylphenol causes precocious mammary gland development in female rat offspring. Journal of Reproduction and Development. 2007;53(2):333-44.[22]
29. El-Dakdoky MH, Helal MA. Reproductive toxicity of male mice after exposure to nonylphenol. Bulletin of environmental contamination and toxicology. 2007;79(2):188-91.

[21] In the study of Laurenzana et al., the tested 4-nonylphenol was reported to consist of 95% branched 4-nonylphenol and 5% branched 2-nonylphenol.
[22] In the study of Moon et al., the tested 4-nonylphenol was reported to consist of 85% branched side-chain isomers.

30. Lu J, Cai D. [Estrogen-like activity of 4-nonylphenol and bisphenol in immature SD rats]. Wei sheng yan jiu = Journal of hygiene research. 2008;37(4):413-6. Article in Chinese, abstract only.
31. Momeni HR, Mehranjani MS, Abnosi MH, Mahmoodi M. Effects of vitamin E on sperm parameters and reproductive hormones in developing rats treated with para-nonylphenol. Iranian Journal of Reproductive Medicine. 2009;7(3):111-6.
32. Peknicova J, Elzeinova F, Zatecka E, Ded L, Dorosh A. The effect of endocrine disruptors on reproductive parameters and expression of selected testicular genes in male mice in vivo. American Journal of Reproductive Immunology. 2012;67:20.
33. Kajta M, Lason W, Litwa E, Rzemieniec J. Behavioral effects of prenatal exposure to 4-para nonylphenol: Studies on female and male mice subjected to the Novel Object Recognition Test. International Journal of Psychiatry in Clinical Practice. 2012;16:37. Conference abstract.
34. Wei Z-m, Xie L-p, Zhu L, Li Z-m, Liang F-d, Yang J-h. Reproductive endocrine toxicity of bisphenol a and 4-nonylphenol in juvenile female cynomolgus. Shiyan Dongwu Yu Bijiao Yixue. 2013;33(5):351-3, 87. Article in Chinese, abstract only.
35. Kawaguchi S, Kuwahara R, Kohara Y, Uchida Y, Oku Y, Yamashita K. Perinatal Exposure to Low-dose Nonylphenol Specifically Improves Spatial Learning and Memory in Male Rat Offspring. Indian journal of physiology and pharmacology. 2015;59(2):211-22.
36. Bistakova J, Forgacs Z, Bartos Z, Szivosne MR, Jambor T, Knazicka Z, et al. Effects of 4-nonylphenol on the steroidogenesis of human adrenocarcinoma cell line (NCI-H295R). Journal of environmental science and health Part A, Toxic/hazardous substances & environmental engineering. 2017;52(3):221-7.

Human studies

37. Chen ML, Chang CC, Shen YJ, Hung JH, Guo BR, Chuang HY, et al. Quantification of prenatal exposure and maternal-foetal transfer of nonylphenol. Chemosphere. 2008;73(1 SUPPL.):S239-S45.
38. Chen GW, Ding WH, Ku HY, Chao HR, Chen HY, Huang MC, et al. Alkylphenols in human milk and their relations to dietary habits in central Taiwan. Food and Chemical Toxicology. 2010;48(7):1939-44.
39. Chen M, Tang R, Fu G, Xu B, Zhu P, Qiao S, et al. Association of exposure to phenols and idiopathic male infertility. Journal of hazardous materials. 2013;250-251:115-21.
40. Chen X, Chen M, Xu B, Tang R, Han X, Qin Y, et al. Parental phenols exposure and spontaneous abortion in Chinese population residing in the middle and lower reaches of the Yangtze River. Chemosphere. 2013;93(2):217-22.
41. Tang R, Chen MJ, Ding GD, Chen XJ, Han XM, Zhou K, et al. Associations of prenatal exposure to phenols with birth outcomes. Environmental Pollution. 2013;178:115-20.
42. Huang YF. Nonylphenol in pregnant women and their matching fetuses: placental transfer and potential risks of infants. Environmental research. 2014;134:143-8.

43. Wang I, Chen M, Chen P. The association of early exposure to phenols and neuro-behavior developemnt in school-aged children. Value in Health. 2015;18(7):A748.
44. Huang YF, Pan WC, Tsai YA, Chang CH, Chen PJ, Shao YS, et al. Concurrent exposures to nonylphenol, bisphenol A, phthalates, and organophosphate pesticides on birth outcomes: A cohort study in Taipei, Taiwan. Science of the Total Environment. 2017;607-608:1126-35.
45. Jamal A, Rastkari N, Dehghaniathar R, Nodehi RN, Nasseri S, Kashani H, et al. Prenatal urinary concentrations of environmental phenols and birth outcomes in the mother-infant pairs of Tehran Environment and Neurodevelopmental Disorders (TEND) cohort study. Environmental research. 2020;184:109331.

10.3 Literature concerning para, ortho or meta 4-nonylphenol (CAS #25154-52-3)

Animal or *in vitro* studies:

1. Yamasaki K, Sawaki M, Noda S, Wada T, Hara T, Takatsuki M. Immature uterotrophic assay of estrogenic compounds in rats given diets of different phytoestrogen content and the ovarian changes with ICI 182,780 or antide. Archives of toxicology. 2002;76(11):613-20.

10.4 Literature concerning nonylphenol which was not further specified

Animal or *in vitro* studies:

1. Colerangle JB, Roy D. Exposure of environmental estrogenic compound nonlyphenol to noble rats alters cell-cycle kinetics in the mammary gland. Endocrine. 1996;4(2):115-22.
2. Shelby MD, Newbold RR, Tully DB, Chae K, Davis VL. Assessing environmental chemicals for estrogenicity using a combination of in vitro and in vivo assays. Environmental health perspectives. 1996;104(12):1296-300.
3. Lee PC, Lee W. In vivo estrogenic action of nonylphenol in immature female rats. Bulletin of environmental contamination and toxicology. 1996;57(3):341-8.
4. Cotroneo MS, Dalu, A., Lamartiniere, C.A., Rodgers, W. . Genistein, but not DDT or nonylphenol, supports growth of surgically induced endometriosis. . Toxicologist 36: 361 (abstract). 1997.
5. Lee PC. Disruption of male reproductive tract development by administration of the xenoestrogen, nonylphenol, to male newborn rats. Endocrine. 1998;9(1):105-11.
6. Lee PC, Arndt P, Nickels KC. Testicular abnormalities in male rats after lactational exposure to nonylphenols. Endocrine. 1999;11(1):61-8.
7. Colerangle JB, Roy D. Exposure of environmental estrogenic compound nonlyphenol to noble rats alters cell-cycle kinetics in the mammary gland. Endocrine. 1996;4(2):115-22.
8. Colerangle JB, Roy D. Exposure of environmental estrogenic compound nonlyphenol to noble rats alters cell-cycle kinetics in the mammary gland. Endocrine. 1996;4(2):115-22.

9. Shelby MD, Newbold RR, Tully DB, Chae K, Davis VL. Assessing environmental chemicals for estrogenicity using a combination of in vitro and in vivo assays. Environmental health perspectives. 1996;104(12):1296-300.
10. Lee PC, Lee W. In vivo estrogenic action of nonylphenol in immature female rats. Bulletin of environmental contamination and toxicology. 1996;57(3):341-8.
11. Cotroneo MS, Dalu, A., Lamartiniere, C.A., Rodgers, W. . Genistein, but not DDT or nonylphenol, supports growth of surgically induced endometriosis. . Toxicologist 36: 361 (abstract). 1997.
12. Lee PC. Disruption of male reproductive tract development by administration of the xenoestrogen, nonylphenol, to male newborn rats. Endocrine. 1998;9(1):105-11.
13. Lee PC, Arndt P, Nickels KC. Testicular abnormalities in male rats after lactational exposure to nonylphenols. Endocrine. 1999;11(1):61-8.
14. Negishi T, Kawasaki K, Suzaki S, Maeda H, Ishii Y, Kyuwa S, et al. Behavioral alterations in response to fear-provoking stimuli and tranylcypromine induced by perinatal exposure to bisphenol A and nonylphenol in male rats. Environmental health perspectives. 2004;112(11):1159-64.
15. Rahimi P, Ahmadi A, Safiei SH, Javan V. Evaluation of nonyl phenol toxic effect on sperm quality of the reproductive system in adult male mice. International Journal of Fertility and Sterility. 2015;9:67. Conference abstract.
16. Kimura N, Kimura T, Suzuki M, Totsukawa K. Effect of gestational exposure to nonylphenol on the development and fertility of mouse offspring. Journal of Reproduction and Development. 2006;52(6):789-95.
17. Chang YC, Wang KL, Hsu RL, Jian CY, Hsia SM, Wang PS. Effects of nonylphenol on the production of testosterone by rat leydig cells. Biology of Reproduction. 2010;83(1). Conference abstract.
18. Lin HW, Jian CY, Chang YC, Huang WJ, Hsu RL, Ho CH, et al. Effects of nonylphenol on the production of progesterone by rat granulosa cells. Biology of Reproduction. 2010;83(1). Article not available, abstract only.
19. Xu J, Wang Y, Yu J, Lu N, Fan QY, Li Y, et al. [Effects of nonylphenol exposure via placenta on early nervous reflex and locomotives of offspring in rat]. Zhonghua lao dong wei sheng zhi ye bing za zhi = Zhonghua laodong weisheng zhiyebing zazhi = Chinese journal of industrial hygiene and occupational diseases. 2010;28(4):272-4. Article in Chinese, abstract only.
20. Jie X, Yang W, Jie Y, Hashim JH, Liu XY, Fan QY, et al. Toxic effect of gestational exposure to nonylphenol on F1 male rats. Birth Defects Research Part B - Developmental and Reproductive Toxicology. 2010;89(5):418-28.
21. Chang LL, Wun WSA, Wang PS. In utero and neonate exposure to nonylphenol develops hyperadrenalism and metabolic syndrome later in life. I. First generation rats (F1). Toxicology. 2012;301(1-3):40-9.

22. Jie Y, Fan QY, Binli H, Biao Z, Zheng F, Jianmei L, et al. Joint neurodevelopmental and behavioral effects of nonylphenol and estradiol on F 1 male rats. International Journal of Environmental Health Research. 2013;23(4):321-30.
23. Chang LL, Wun WSA, Wang PS. Recovery from developmental nonylphenol exposure is possible for female rats. Chemico-Biological Interactions. 2014;221:52-60.
24. Keikha L, Shariatzadeh MA, Naderi Noreini S. Evaluation of the protective effect of Nigella sativa oil on testicular tissue in mice following exposure to para-nonylphenol. Iranian Journal of Reproductive Medicine. 2014;12(6):99-100. Article in Arabic, abstract only.
25. Yu J, Luo Y, Yang XF, Yang MX, Yang J, Yang XS, et al. Effects of perinatal exposure to nonylphenol on delivery outcomes of pregnant rats and inflammatory hepatic injury in newborn rats. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas. 2016;49(12):e5647.
26. Di QN, Cao WX, Xu R, Lu L, Xu Q, Wang XB. Chronic low-dose exposure of nonylphenol alters energy homeostasis in the reproductive system of female rats. Toxicology and Applied Pharmacology. 2018;348:67-75.

Human studies

27. Ademollo N, Ferrara F, Delise M, Fabietti F, Funari E. Nonylphenol and octylphenol in human breast milk. Environment international. 2008;34(7):984-7.
28. Chang CH, Chen ML, Liao KW, Tsai YA, Mao IF, Wang TH, et al. The association between maternal nonylphenol exposure and parity on neonatal birth weight: A cohort study in Taiwan. Chemosphere. 2013;93(6):1145-52.
29. Yum T, Lee S, Kim Y. Association between precocious puberty and some endocrine disruptors in human plasma. Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering. 2013;48(8):912-7.
30. Chang CH, Tsai MS, Lin CL, Hou JW, Wang TH, Tsai YA, et al. The association between nonylphenols and sexual hormones levels among pregnant women: A cohort Study in Taiwan. PLoS ONE. 2014;9(8).
31. Knez J, Kovacic B, Reljic M, Vlaisavljevic V. Environmental exposure to selected endocrine disrupting chemicals adversely influences reproductive hormone levels in men. Human Reproduction. 2015;30:i7-i8. Article not available, abstract only.
32. Lin CC, Chien CJ, Tsai MS, Hsieh CJ, Hsieh WS, Chen PC. Prenatal phenolic compounds exposure and neurobehavioral development at 2 and 7 years of age. Science of the Total Environment. 2017;605-606:801-10.

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