TOXICOLOGICAL PROFILE FOR NONYLPHENOL

September 2009

Integrated Risk Assessment Branch
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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Executive Summary

This toxicological profile on **nonylphenol (NP)** describes its effects freshwater and marine life, humans, and laboratory animals. In recent years, NP and related chemical forms of NP have raised concerns because of their effects on the endocrine system, the organ system producing hormones that regulates many of the body's functions. Such concerns have led to many restrictions on the use of NPs within the European Union.

Use and Exposure

NP is a synthetic organic chemical produced in relatively large quantities in the United States. Primary uses include: (1) a building block of nonionic surfactants (i.e., agents that reduce surface tension of liquids) used in lubrication, defoaming agents, scouring fibers, emulsifiers, wetting and de-wetting agents, dyes, and other products; and (2) a component in a stabilizer used in plastics and vulcanized rubber.

NP-surfactants are the principal source of NP release into the environment. Aquatic anf marine life exposure occurs when the substantial quantities of NP-surfactants, discharged into wastewater, biodegrade into several by-products, including NP. While not a significant source of NP in the environment, unreacted NP in plastic may result in direct human exposures when the chemical leaches out of plastic in close contact with foods.

Environmental Occurrence

Due to its physical-chemical characteristics, NP accumulates and persists in sewage sludge, river sediments, and other environmental compartments. The occurrence of NP in the environment is clearly correlated with human activities such as wastewater treatment, land filling and sewage sludge recycling.

NP has been reported at concentrations of up to 0.89 micrograms per liter (µg/L) in freshwater bodies, over 1 µg/L in municipal treatment plant effluents, and up to 2.76 µg/L in coastal waters. Most adverse effects reported in laboratory experiments occur at concentrations above 1 µg/L. More data on environmental concentrations of NP in marine systems will allow a more complete assessment of the impacts of environmental levels of NP on marine organisms.

Effects on Aquatic Life

Laboratory studies indicate that NP can induce a variety of reproductive effects in aquatic life, including fish and shellfish. Reported reproductive effects include:

- Changes in male and female hormone levels in turbot
- Decreased gamete production and fertilization in medaka and zebrafish
- Reduced hatching of rainbow trout embryos
- Altered sex ratios in offspring of NP-exposed oysters
- Development of intersex trout, bream, and frogs (i.e. offspring with characteristics of both sexes)

NP can also induce a variety of non-reproductive effects, such as the inability to maintain fluid and electrolyte balance in sea bream and Atlantic salmon, which could prevent their migration

from fresh water to sea water. Clams and sea urchins exposed to NP have exhibited decreased respiration and increased malformations, respectively.

Health Hazard and Toxicity in Humans and Laboratory Animals

- **Reproductive and Developmental Effects.** NP can act as an estrogen, a group of naturally occurring steroid compounds that function as the primary female sex hormone. Sufficient evidence was found to show that NP causes reproductive effects in laboratory animals. These effects, which are thought to be linked to NP's estrogenic activity, include:
  - Lowered levels of the male sex hormone testosterone
  - Effects on the testes, including decreased sperm production
  - Increased uterine weight, suggesting that NP may affect female reproduction
  - Altered development of the brain region responsible for male and female behavior
  - Hyperactivity in juvenile animals and animals exposed before birth due to effects on the development of regions of the brain.

  In humans, limited information is available on possible reproductive effects. One study reported early onset puberty in children exposed to NP while *in utero*.

- **Cancer.** There is no information on whether NP is carcinogenic in laboratory animals or in humans.

- **Immune and Thyroid Effects.** There is some evidence that NP affects the immune system in laboratory animals and limited evidence that it affects thyroid function and obesity. Many if not all of these effects appear to be related to NP's estrogen-like effect and its ability to disrupt the endocrine system.

- **Nervous System Effects.** Prenatal exposure of laboratory rodents to NP results in neurobiological alterations, including some sexually dimorphic behaviors. Studies conducted with cultured cells and tissues suggest that NP could adversely affect brain development and may cause neurodegeneration.

Summary Table

This table provides some idea of the availability of information on the toxicology of NP for the endpoints and organisms identified. It also provides some sense of the evidence available in that information can be used to determine if the endpoint effect does or does not occur. If there is no information the evidence column will be marked with a "--."

Health Effect	Human		Lab Animal		Aquatic Life	
	Information	Evidence	Information	Evidence	Information	Evidence
Reproductive						
male	L	L	Su	Su	Su	Su
female	L	L	S	S	Su	Su
Developmental	S	S	S	S	Su	Su
Neurological	N	--	S	S		
Cancer	N	--	N	--	N	--
Immunological	N	--	S	S	N	--
Other Chronic effects					Su	Su
Thyroid	N	--	L	L		
Obesity	N	--	L	L		
Acute					Su	Su

N = None S = Some
L = Little Su = Sufficient
These rating categories are qualitative in nature and designed to give the reader a general sense or the availability and strength of the information.

Abbreviations

ADHD	attention-deficit hyperactivity disorder
AGD	anogenital distance
ANOVA	analysis of variance
ATPase	adenosine triphosphatase
AWQC	ambient water quality criteria
BCF	bioconcentration factor
11 β-HSD	β-hydroxysteroid dehydrogenase
cAMP	cyclic adenosine monophosphate
CERHR	Center for the Evaluation of Risks to Human Reproduction
CG	chorionic gonadotropin
Con A	concanavalin A
D	dopamine receptors (D_1 or D_2)
DART	developmental and reproductive toxicities
DNA	deoxyribonucleic acid
dph	days post-hatch
E2	17 beta-estradiol
EC_{50}	median effective concentration
2-EH	2-ethylhexanol
ER	estrogen receptor
ERL	environmental risk limit
FasL	Fas ligand
FSH	follicle-stimulating hormone
hCG	β-human chorionic gonadotropin
hES	human embryonic stem
HPOA	hypothalamic/preoptic area
ICI	ICI 182780 (Faslodex) from AstraZeneca
IFN	interferon
IgE	immunoglobulin E
IL	interleukin
K_{OW}	Octanol-water coefficient
LC_{50}	lethal concentration to 50% of the population
LD_{50}	lethal dose to 50% of the population
LH	luteinizing hormone
LOEC	lowest observed concentration
LOELs	lowest observed effect levels
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinases
MIF	migration inhibitory factor
MIP-1α	macrophage inflammatory protein-1α
µM	micromolar
mRNA	messenger ribonucleic acid
NADPH	β-nicotinamide adenine dinucleotide phosphate
NF	nuclear factor
NGF	nerve growth factor

NIS	sodium/iodide symporter
nM	nanomolar
NO	nitric oxide
NOEC	no observed effect concentration
NOELs	no observed effect levels
NP	nonylphenol
NSC	neural stem cells
OEHHA	Office of Environmental Health Hazard Assessment
OPC	California Ocean Protection Council
PCBs	polychlorinated biphenols
PDI	protein disulfide isomerase
p.f.	post-fertilization
PKC	protein kinase C
PND	postnatal day
PPAR-γ	peroxisome proliferators-activated receptor-γ
PPB	parts per billion
Ppm	parts per million
PVC	polyvinyl chloride
qTR LBD	ligand-binding domain of thyroid hormone receptor beta
qTTR	Japanese quail transthyretin
SDN-POA	sexually dimorphic nucleus of the medial preoptic area
SEB	Staphylococcus enterotoxin B
SHBG	sex hormone-binding globulin
T3	triiodothyronine
$^{[125I]}$T3	3,3',5-L-$^{[125I]}$triiodothyronine, iodine isotpe labeled
T4	thyroxine
TH	thyroid hormone
Th	T helper cell
TNF-alpha	tumor necrosis factor-alpha
TSH	thyroid stimulating hormone
VTA	Ventral Tegmental Area
VTG	vitellogenin

Introduction

On February 8, 2007, the California Ocean Protection Council (OPC) passed a resolution, "On Reducing and Preventing Marine Debris." Scientists are investigating whether constituents leach out of plastic products in the marine environment and present a threat to the health of wildlife and humans. The OPC has asked the Office of Environmental Health Hazard Assessment (OEHHA) to prepare toxicity profiles characterizing certain chemical constituents of plastics that are thought to be harmful to marine life and humans. In preparing this profile, OEHHA reviewed reported information on the adverse effects of exposure to NP in aquatic organisms in the laboratory and in the natural environment, humans, and experimental laboratory animals.

Properties and Uses

Nonylphenol (NP) (CAS number, 104-40-05) is a product of industrial synthesis formed during the alkylation process of phenols (ring structure in Figure 1). The addition of ethoxyl groups to the parent compound produces nonylphenol ethoxylates (NPE), which are used to produce industrial surfactants. Alkylphenol ethoxylates are the second largest group of nonionic surfactants in commercial production, of which NPEs account for approximately 80 percent. NP, the predominant environmental biodegradation product of NP ethoxylates, is ubiquitous and moderately persistent. Physical properties and environmental fate have been reviewed by the U.S. Environmental Protection Agency (USEPA, 2005).

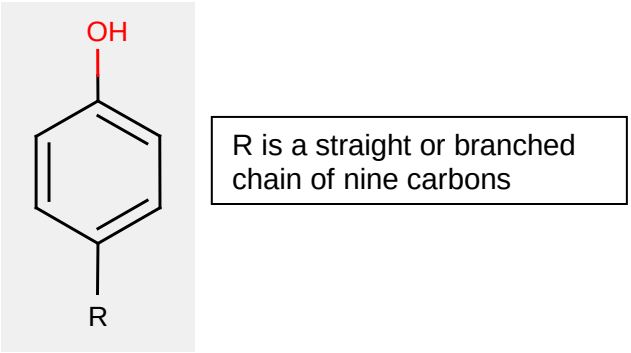

Figure 1: NP structural formula

Table 1: Nonylphenol Properties (based on USEPA (2005)

PROPERTY	VALUE	UNITS
Molecular weight	220	g/mole
Empirical formula	$C_{15}H_{24}O$	
Specific gravity	0.953	g/mL
pKa	10.7±1.0	
Solubility, pH 7	4.6	mg/L
Solubility, pH 9	6.2	mg/L
Solubility, pH 11	11.9	mg/L
Solubility, sea water	3.6	mg/L
Log K_{OW}	3.8 – 4.8 (4.5)	
T ½ in water w/ POTW sludge	8.2	Days
Vapor pressure	0.00455 ± 0.0035	Pa

NPEs are widely used as detergents, emulsifiers, and surfactants (wetting agents) in household and industrial products such as paints, plastics, cosmetics, lubricant oils, construction materials, vulcanized rubber, and paper. They are also used in the processing of fuels, metals, petroleum, textiles, agricultural chemicals, and leather. Substantial quantities of NP-containing compounds reach sewage treatment works, where they biodegrade into several by-products, including NP. Due to its physical-chemical characteristics, such as low water solubility, NP accumulates in environmental compartments like sewage sludge and river sediments, where it is moderately persistant.

Unreacted NP in the stabilizer used in plastic may leach out of the plastic; while this is not a significant source of NP in the environment, it can be a direct route of exposure if the plastic is in close contact with foods or if the plastics are ingested by aquatic organisms. The occurrence of NP in the environment is clearly correlated with human activities such as wastewater treatment, land filling and sewage sludge recycling.

Environmental Criteria and Contamination

USEPA (2005) summarized aquatic toxicity data through early 2003. They established Ambient Water Quality Criteria (AWQC; summarized in Table 2) and concluded that NP is an estrogen agonist (NP can act like the female hormone estrogen in an organism).

Table 2: EPA Ambient Water Quality Criteria*

AWQC, FW (one-hour average)	***28 µg/L***
AWQC, FW (four-day average)	***6.6 µg/L***
AWQC, SW (one-hour average)	***7 µg/L***
AWQC, SW (four-day average)	***1.7 µg/L***

Not to be exceeded more than once every 3 yrs. Criteria are based on whole-animal effects like reproduction; i.e. they do not consider biochemical changes in the absence of whole-animal effects.* *(USEPA, 2005)****. FW=fresh water; SW=salt water.*

Sources and environmental concentrations have been reviewed by USEPA (2005). Sources and environmental concentrations found in various parts of the world are summarized in Table 3.

TABLE 3: Nonylphenol levels in water and sediment

Water (µg/L)	Sediment (µg/kg)	Country	Reference
	<1 - 6760	Korea	(Tanaka and Nakanishi, 2002)
0-2.7 (municipal effluents)		Japan	(Komori et al., 2006)
0.0003 – 0.0025 sea		Japan	(Hashimoto et al., 2005)
0.001 – 0.004 fresh	2 - 12	Japan	(Hashimoto et al., 2005)
0.20 – 2.76 sea		Singapore	(Basheer et al., 2004)
n.d.–0.89; mean 0.031		Austria	(Bursch et al., 2004)
20% >1 µg/l; some > 6 µg/l (POTW effluent)		Canada	(Berryman et al., 2004)
0.5 - 15	20 - 640	Spain	(Petrovic et al., 2002)
2.13 (POTW influent) 0.32 (POTW effluent)		Germany	(Korner et al., 2000)
4.9		Taiwan	(Hong and Li, 2007)
6–7 (municipal effluents)		Canada	(Fernandez et al., 2007)
5	180,000	Laboratory	(Bettinetti et al., 2002)
20	600,000	Laboratory	

Environmental Fate, Transport, and Bio-uptake

Nonylphenol is taken up from water and sediment by aquatic biota. It can accumulate in the tissues of these organisms, but does not accumulate to the degree that NP's K_{OW} (octanol-water coefficient) would suggest based just on its lipid solubility. It can move up the food chain, but does not biomagnify to any great degree.

NP transfer from sediment into benthic worms was inversely related to sediment organic carbon content (F_{OC}). Algae grown in medium containing 100 µg NP/L accumulated up to 917 µg NP/g of algae, indicating that NP has a higher affinity for the algae than water. *Artemia franciscana* (brine shrimp) fed the treated algae grew faster than artemia fed control algae, but accumulated only trace amounts of NP. Zebrafish fed the treated artemia did not show any significant differences in growth, reproduction, cytochrome P450 activity, superoxide dismutase activity and vitellogenin (VTG) levels (Correa-Reyes et al., 2007).

Female roach fish exposed to a NP concentration of 4.9 µg/L radio-labelled technical NP over a 4-day period exhibited apparent bioconcentration factors (BCF) of 34,121 and 605, in bile and liver, respectively; in other tissues, apparent BCF values were recorded between 13 and 250 (Smith and Hill, 2004). This suggests that NP is metabolized and excreted in the bile. NP accumulated in the liver, gill, skin, gut, fat, and kidney tissue of trout (Ahel et al., 1993; Coldham et al., 1998; Lewis and Lech, 1996) as well as shrimp, mussels, and stickleback fish (Ekelund et al., 1990). NP has also been found in high levels in seafood from Singapore, especially prawns (Basheer et al., 2004) and at even higher levels in field-collected mussels, clams, and squid from Italy (Ferrara et al., 2001) (Table 4). These data indicate a potential pathway for human exposure through consumption of market seafood items.

The log K_{OW} of nonylphenol ranges from 3.80 to 4.77, indicating that moderate bioaccumulation in aquatic organisms may be expected (USEPA, 2005). NP accumulated in the liver, gill, skin, gut, fat, and kidney tissue of trout (Ahel et al., 1993; Coldham et al., 1998; Lewis and Lech, 1996) as well as shrimp, mussels, and stickleback fish (Ekelund et al., 1990). NP has also been found in high levels in seafood from Singapore, especially prawns (Basheer et al., 2004) and at

even higher levels in field collected mussels, clams, and squid from Italy (Ferrara et al., 2001) (Table 4). These data indicate a potential pathway for human exposure through consumption of market seafood items.

Reported laboratory bioconcentration factors (BCFs) and field-derived bioaccumulation factors (BAFs) do not support the expected accumulations in tissues, indicating that some nonylphenol is metabolized (USEPA, 2005). A single major metabolite of NP was present in liver and bile of the female roach exposed to a NP concentration of 4.9 µg/L over four days. The metabolite was identified as the glucuronide conjugate of 4-(hydroxy-nonyl)-phenol (Smith and Hill, 2004). Similarly, NP was metabolized by hepatic cytochrome P450 enzymes in the rainbow trout (*Oncorhynchus mykiss*) and bile from the fish contained the glucuronic acid conjugates of nonylphenol; thus, bile may be a major route of nonylphenol excretion (USEPA, 2005).

Table 4: Bio-uptake and Bioconcentration in Aquatic Organisms

Species	Water (µg/L)	Tissue (µg/kg)	BCF	Reference
Blue mussel (*Mytilus* sp.)			1.4 – 7.8	(USEPA, 2005)
Atlantic salmon (*Salmo salar*)			75	
Marine amphipods			46 – 185	
Algae			487	
Shrimp (*Crangon crangon*)			90 – 110	(Ekelund et al., 1990)
Mussels (*Mytilus edulis*)			2740 – 4120	
Stickleback fish (*Gasterosterus aculeatus*)			1200 –1300	
periphyton	0.1 – 0.4	8-130	160 – 650	(Takahashi et al., 2003)
benthos	0.1 – 0.4	8-140	63 – 990	
Mussels	<0.0005– 0.21	131 – 211	1000	(Pojana et al., 2007)
Medaka (*Oryzias latipes*) eggs	62	2000-7000	30 – 100	(Ishibashi et al., 2006)
Prawn (*Penaeus monodon*)	0.20 – 2.76	197.0 ± 13.1		(Basheer et al., 2004)
Crab (*Portunus pelagicus*)		103.1 ± 36.0		
Blood cockle (*Anadara granosa*)		54.0 ± 6.1		
White clam (*Meretrix meretrix*)		46.6 ± 11.4		
Squid (*Loligo* sp.)		64.8 ± 13.7		
Indian scad fish (*Decapterus russelli*)		60.5 ± 10.4		
Mussel (*Mytilus galloprovincialis*)		260		(Ferrara et al., 2001)
Clam (*R. decussates* and *C. gallina*, pooled)		248		
Squid (*Loligo vulgaris*)		512		

Species	Water (µg/L)	Tissue (µg/kg)	BCF	Reference
Cuttlefish (*Sepia officinalis*)		240		
Great pond snail (*Lymnaea stagnalis*)	99 – 124	69-266	1 – 2.5	(Lalah et al., 2007)
	104	23548	242	(Lalah et al., 2003)
Blackworm (*Lumbriculus variegates*)			1.8 – 33.6	(Maenpaa and Kukkonen, 2006)
Blue mussels (*Mytilus edulis*)	1985	4.0		(Gunther et al., 2001)
	1995	1.1		
Common carp (*Cyprinus carpio*)		100-200	280	(Mitchelmore and Rice, 2006)
Alga (*Isochrysis galbana*)	100	917,000	9170	(Correa-Reyes et al., 2007)
Rainbow trout (*Oncorhyncus mykiss*)	66	140	2.1	(Uguz et al., 2003)
	220	1280	5.8	
	660	1370	2.1	

Toxicology: Marine and Other Aquatic Organisms

Many of nonylphenol's effects on aquatic organisms are attributed to its estrogenic activity, but it also causes toxic effects that are not obviously related to its estrogenic activity, such as effects on growth, behavior, respiration, and osmoregulation. As noted in the summary table, there is sufficient qualitative information on acute, chronic, reproductive and developmental toxicity of NP to aquatic organisms. There is no information on immunotoxicology or carcinogenicity of NP.

Reproductive and Developmental Toxicity

NP has been found to affect various indicators of reproduction in aquatic organisms at concentrations ranging from 0.13 µg/L up to the milligram/liter range. NP competes with estrogen for binding to the estrogen receptor (Danzo, 1997; Flouriot et al., 1995), thereby affecting reproduction and development (Christiansen et al., 1998; Colborn et al., 1993). The most serious and widespread environmental consequences of exposure to NP are likely to be related to its estrogenic activity rather than direct lethality (Lech et al., 1996). Because steroid hormone balance is thought to affect the development of the brain–pituitary–gonad axis in fish, adverse reproductive effects may potentially arise in juvenile males exposed chronically to such xenobiotics (i.e. exogenous chemicals and in this case with estrogenic activity) (Burkhardt-Holm et al., 2000). NP disrupts steroid hormone balance in juvenile turbot, with males being much more sensitive to this effect than females: NP induced a decrease of the androgen-to-estrogen ratio in testis, plasma, and bile and depressed androgen levels, perhaps as a consequence of 11b-hydroxylase or 11b-hydroxysteroid dehydrogenase activity disturbance, (Labadie and Budzinski, 2006).

Vitellogenin (VTG) Assay in Fish

A useful way to determine the estrogenicity of a xenobiotic in fish is through a specific assay for vitellogenin (VTG). The VTG assay is a frequently used *in vivo* biomarker for estrogenicity in egg laying vertebrates (Heppell et al., 1995; Lattier et al., 2001). VTG is a large, complex,

calcium-binding phospholipoglycoprotein that is required for normal oocyte (prefertilized egg cell) maturation in developing female fish (Matozzo et al., 2007). It is produced in the liver in response to estrogen stimulation, secreted in the blood, and transported to the oocyte, where it is incorporated as constituents of the yolk. Being estrogen-dependent, VTG production is normally restricted to females; little if any VTG can be detected in males or sexually immature females. However, males do carry the VTG gene and exposure to estrogens can trigger its expression (Sumpter and Jobling, 1995). VTG can be measured in the liver, blood, and mucus from male and female fish as well as in primary hepatocyte cultures (Navas and Segner, 2006).

Increased VTG synthesis resulting from NP exposure has been reported in flounder (Kirby et al., 2007), cod (Larsen et al., 2006), rainbow trout (Ackermann et al., 2002), fathead minnow (Brian et al., 2005), atlantic salmon (Meucci and Arukwe, 2006), killifish (Garcia-Reyero et al., 2004), medaka (Ishibashi et al., 2006; Lee et al., 2003), sheepshead minnow (Hemmer et al., 2002), and mysid shrimp (Ghekiere et al., 2006). Male carp in the Cuyahoga River near the outfall of the city of Akron's wastewater treatment plant had higher tissue NP levels and about twice the serum VTG levels compared to carp from up-river sites. Serum VTG was correlated with tissue NP levels ($r = 0.30 – 0.97$) (Mitchelmore and Rice, 2006). Pickford et al. (2003) estimated that NP absorbed from the water was 10 times more potent in inducing VTG in male fathead minnows than NP administered in the diet.

VTG induction is a marker of estrogenic exposure, but it has also been correlated with other endpoints. Turbot exposed to 29 µg/L NP exhibited increased VTG and zona radiata protein (protein subunits of the inner eggshell) (Larsen et al., 2006) as well as decreased plasma, testicular, and biliary testosterone, androstenedione, and β estradiol (Labadie and Budzinski, 2006; Martin-Skilton et al., 2006). Dose-dependant plasma VTG production was the most sensitive biomarker of exposure to NP in male killifish (*Fundulus heteroclitus*) (Pait and Nelson, 2003). Medaka exposed to NP concentrations >11.6 µg/L had increased hepatic VTG levels and their offspring showed altered sex ratios and formation of testis-ova (Seki et al., 2003; Yokota et al., 2001). High concentrations of plasma VTG and an increased prevalence of ovotestes (gonads with both testicular and ovarian aspects) occurred in wild male bream in a small river receiving effluent from a large sewage treatment plant. After employing *in vitro* and *in vivo* bioassays the authors concluded that hormones (especially 17a-ethynylestradiol) and possibly also NP ethoxylates are primarily responsible for these effects (Vethaak et al., 2005).

Four months prior to spawning, adult rainbow trout of both sexes were exposed intermittently to NP concentrations of 1 and 10 µg/L. VTG levels in the plasma of adult male rainbow trout were significantly increased compared to the control group. Embryo mortality was increased in both treatment groups, while hatching rates were significantly reduced in the 10 µg NP/L group. VTG levels were significantly higher in female offspring than in the controls, but there was no alteration in sex ratios. Occasionally, intersex occurred in both male and female offspring of exposed fish and estradiol was increased by two-fold in the plasma of male offspring and testosterone by 13-fold in the plasma of female progeny. These hormonal imbalances in the offspring of exposed fish indicate a transgenerational endocrine disruption (Schwaiger et al., 2002).

Other Reproductive Endpoints in Fish

NP-exposed cod had higher metabolism rates of estradiol in their livers (Martin-Skilton et al., 2006). NP-exposed turbot had lower ovarian P450 aromatase (an enzyme that converts

testosterone to estradiol), lower levels of testosterone and estradiol in plasma, and lower metabolism rates of sex steroids than those from the control group (Martin-Skilton et al., 2006). In medaka, decreased fecundity (ability to reproduce) and fertility (Ishibashi et al., 2006; Kang et al., 2003), increased ratio of females to males and mixed sex characteristics (Balch and Metcalfe, 2006; Kang et al., 2003), and decreased ratio of motile spermatozoa and decreased spermatozoa swimming speed (Hara et al., 2007) have been reported. Semen volume was reduced in rainbow trout exposed to a NP concentration of 0.13 µg/L, and embryo survival and development were reduced at concentrations of 0.28 to 0.75 µg/L (Lahnsteiner et al., 2005).

Zebrafish (*Danio rerio*) exposed to ≥100 µg/L NP from 2 to 60 days post-hatch (dph) had concentration-dependent suppression of gametogenesis (formation of the mature egg and sperm) in both males and females. NP concentrations of 10 µg/ml and higher caused enlargement of Sertoli cells and significantly stimulated DNA replication and mitosis of type A spermatogonia in cultured Japanese eel (*Anguilla japonica*) testicular cells. However, these spermatogonia did not further develop unless 11-ketotestosterone was added to the culture medium (Miura et al., 2005).

Exposure to NP ≥100 µg/L (nominal) from 2 to 60 days post hatch caused concentration-dependent suppression of gametogenesis in both male and female zebrafish. Some recovery was indicated by histologically normal testes after fish were placed in clean water from 60 to 300 dph. In females, however, recovery was incomplete at 300 dph (Weber et al., 2003). Brian et al. (2005) demonstrated the potential for estrogenic chemicals to act additively at environmentally relevant concentrations. Using VTG induction in male fathead minnows as an endpoint, they showed that the combined effects of a mixture of five estrogenic chemicals, estradiol, ethynylestradiol, NP, octylphenol, and bisphenol A—each chemical at one-fifth of its median effective concentration (EC_{50})—were consistent with the effects predicted by concentration addition.

NP exposure was associated with increased intersex frogs, altered sex ratios, and increased gonadal development (Mackenzie et al., 2003). African clawed frog (Xenopus laevis) embryos were exposed to eight different concentrations of NP, from 3 to 96 hours post-fertilization (p.f.). Short body length, microcephaly, flexure, edema, and abnormal gut coiling were induced by 20µM NP by 96 hours p.f. (Sone et al., 2004). Development was delayed in *Rana catesbeiana* tadpoles (Christensen et al., 2005).

Reproductive Effects on Invertebrates

There are many reports of reproductive impairment in invertebrates exposed to NP concentrations >50 µg/L; several studies report reproductive effects at concentrations between 1 and 50 µg/L, and there are a few reports of reproductive effects at concentrations <1 µg/L. It was lethal to some tubifex worms

at concentrations of 600 µg/g sediment. Those that survived this concentration had empty spermatheca (a female tubifex worm organ that receives sperm from the male). If present, spermatic sacs had few germinal elements and no spermatozons. Ovaries were present but ovocytes were not developed (Bettinetti and Provini, 2002). *Lymnaea* sp. snails exhibited decreased fecundity at 100 µg/L (Czech et al., 2001), along with decreased egg masses, increased embryo mortality, and delayed development (Lalah et al., 2007). A NP concentration of 0.2 µg/L was toxic to sea urchin sperm, resulting in reduced fertilization (Ghirardini et al., 2001). Development was delayed in copepods at concentrations as low as 0.1 µg/L (Marcial et

al., 2003). NP exposure also resulted in fewer mudsnail embryos (Duft et al., 2003). Altered sex ratios were reported in chironomids exposed to 1 µg/L (Lee and Choi, 2006) and decreased fecundity in daphnids at concentrations between 25 and 50 µg/L (Brennan et al., 2006). The 96-hr LC_{10} for *Hydra attenuata* was 20 µg/L, making it one of the most sensitive freshwater invertebrate species behind the amphipod *Hyalella azteca* (Pachura-Bouchet et al., 2006; Pachura et al., 2005).

A 48- hour exposure of oyster (*Crassostrea gigas)* larvae to NP at postfertilization days 7–8 resulted in significant alterations to the sex ratio towards females and a 30 percent incidence of fully functional hermaphroditism. Transgenerational effects included poor offspring survival rates, delayed larval growth rates, and inhibition of settlement and metamorphosis. Gamete viability was also affected, resulting in poor embryonic and larval development (up to 100 percent mortality) of the subsequent generation. Three days' exposure to 1 or 100 µg/L NP resulted in a 70 percent or 90 percent reduction in oysters with motile sperm, respectively (Nice, 2005).

Transgenerational effects were not detected in the freshwater water flea (*Daphnia galeata)*. The population-level EC_{50}, the concentration of NP that reduced the intrinsic rate of natural population increase by 50 percent, was estimated as 65.2 µg/L for the first generation and 81.5 µg/L for the second generation. The 48-h LC_{50}, 60.8 µg/L, is a good indicator of the chronic population-level effects of this chemical to this species (Tanaka and Nakanishi, 2002).

Other Types of Toxicity

Other Toxicity in Fish

NP can interfere with osmoregulation. Sea bream had decreased renal sodium-potassium dependent ATPase and increased plasma osmolality (Carrera et al., 2007). Atlantic salmon smolts showed decreased sodium-potassium dependent ATPase in the gills, decreased ability to adapt to sea water, increased plasma cortisol, lower plasma insulin-like growth factor, decreased T_3, and, at higher concentrations, complete loss of osmoregulatory control and death (Arsenault et al., 2004; Lerner et al., 2007a; Lerner et al., 2007b). Zebrafish (*Danio rerio*) exposed to $\geq$10 µg/L NP from 2 to 60 dph had renal lesions including pyknotic nuclei in tubular and interstitial (hematopoietic) cells. Some recovery was indicated by histologically normal kidneys after fish were placed in clean water from 60 to 300 dph (Weber et al., 2003).

Juvenile rainbow trout exposed to 220 mg NP/L for up to 28 days appeared healthy, but had histopathological changes in their livers, which also showed an increase in the activity of glutathione-S-transferase. All juvenile rainbow trout exposure to 660 mg NP/L died after 4 days (Uguz et al., 2003). Other effects in fish include increased micronuclei in turbot (Barsiene et al., 2006), vacuolation of rainbow trout epidermal cells (Burkhardt-Holm et al., 2000), increased mortality in medaka (Ishibashi et al., 2006), increased mortality and decreased weight and length in platyfish (Magliulo et al., 2002), increased cytochrome P_{450} (CYP19A2) transcription (Kazeto et al., 2004), and decreased plasma insulin-like growth factor and growth of chinook salmon (Fernandez et al., 2007). Fifteen days' exposure to 30 ppb NP caused a decrease in 7-ethoxyresorufin O-deethylation and cytochrome P_{450} (CYP1A) activity and a decrease in glutathione in juvenile Atlantic cod (Sturve et al., 2006).

Other Toxicity in Invertebrates

Midge larvae exposed to NP exhibited increased heat-shock protein mRNA, increased glutathione-S- transferase, (Lee and Choi, 2006; Lee et al., 2006), DNA strand breaks at NP concentrations of ≥0.045 µM (9.9 µg/L), with a marginal effect at 0.005 µM (1.1 µg/L) (Park and Choi, 2007) and increased hemoglobin mRNA (Lee and Choi, 2006). LeBlanc (2000) observed abnormal water flea embryos. Development was delayed in copepods (Forget-Leray et al., 2005). Aquatic microcosms and mesocosms demonstrated changes in zooplankton and phytoplankton species composition (Hense et al., 2003).

Decreased respiration, decreased absorption efficiency, decreased superoxide mutase activity, decreased re-burrowing, and destabilation of hemocyte lysosomal membranes have been reported in bivalves (Canesi et al., 2007; Matozzo and Marin, 2005). Malformed sea urchins have been reported (Cakal Arslan and Parlak, 2007).

Summary and Aquatic Hazard Assessment

NP is toxic to a wide variety of marine and freshwater vertebrate and invertebrate species in laboratory settings. Toxic effects include reproductive and endocrine effects as well as general and systemic toxicity. Most effects are associated with concentrations ranging from 1 to 1000 µg/L, but there are some reports of effects at environmental concentrations less than 1 µg/L. Since most environmental concentrations are less than 1 µg/L, it appears that only the most vulnerable species are likely to be affected and only at the upper range of environmental concentrations. A summary of aquatic toxicity can be found in Appendix 1.

Unfortunately, most of the environmental concentration data are from fresh water systems. It would be useful to gather data on levels in marine environments, especially near municipal and industrial outfalls, landfills, and other possible point sources of NP. Although extrapolating the results of laboratory studies to environmental settings is common practice, it would be preferable to have data based on free-living marine organisms. Unfortunately, there is a paucity of these more-difficult studies.

Animal and Human Studies

Reproductive Toxicity

Introduction

Reproductive toxicity encompases the adverse effects of a substance on the reproductive ability of male and female organisms. Developmental toxicity, discussed in the section following below, is a subset of reproductive toxicity. Reproductive toxicity studies on laboratory animals and *in vitro* studies on human cell lines may be helpful in understanding the mechanism by which NP can alter reproduction in marine organisms, particularly marine mammals. Laboratory animal studies and *in vitro* studies are controlled studies—dose, routes of exposure and length of exposure are all known—which allows for postulation on what minimum levels of exposure exert effects and possibly postulation on the mechanism by which NP might alter reproductive function.

Because the bulk of the literature available is toxicological studies in laboratory rodents, some consideration should be given to the relevance of the findings to marine life. NP is "estrogenic" and may act through the estrogen receptor (ER). A number of ERs have been identified in mammals. This family of nuclear receptors is present in all known vertebrates (Thornton, 2001).

Invertebrates have a variant ER (based on DNA sequencing), which does not, however, bind estrogen.

Laboratory Animal Studies

Males

The varied effects of NP—an exogenous (i.e. originating outside the organism) estrogenic compound—has been extensively examined in laboratory rodents. The effects of NP on numerous endpoints such as reproductive organ characteristics and weight, characteristics of spermatozoa, and hormone profiles have been studied at various dose levels.

Oral exposure of male Sprague-Dawley rats from postnatal day (PND) 23 to PND 52 - 53 with 100 mg NP/kg body weight resulted in testicular damage. Testicular tube diameter was significantly decreased, and 5 out of 12 rats from the NP-treated group did not show any form of spermatogenic cycle (Tan et al., 2003). A number of *in vivo* studies examined the testicular effects of NP, although there was no apparent apoptosis (ie: cell death) in the Sertoli cells. However, several studies using *in vitro* methods showed NP can cause dramatic changes in rat Sertoli cells. An *in vitro* study demonstrated NP induced apoptosis of rat Sertoli cells. Sertoli cells from 20 day old Sprague-Dawley rats were cultured at a density of 5.0×10^4 cells/90 μl; 10 μl of medium containing NP was added such that NP concentrations in 100 μl medium were 0, 200, 1,000, 3,000, or 5,000 parts per billion (ppb) (Wang et al., 2003). NP exposure induced a concentration- and time-dependent decrease in Sertoli cell proliferation. At 3,000 ppb, proliferation was significantly decreased after 72 hours of exposure (Wang et al., 2003). At 5,000 ppb, Sertoli cell proliferation was significantly decreased as early as 24 hours, and was further inhibited at 48 and 72 hours (Wang et al., 2003). Lee et al. demonstrated treatment of neonatal Sprague-Dawley male pups with 8.0 mg NP/kg body weight for 15 days after birth caused changes in the histology (increased intertubular spaces) of the testes when examined on 31 days of age and at approximately 8 months of age (Lee et al., 1999).

Neonatal exposure of Alpk:APfSD (Wistar derived) male rat pups to 8 mg NP/kg/day on PND 1 to PND 10 via intraperitoneal injection produced no significant effect on the male reproductive tract (Odum and Ashby, 2000). NP did not appear to affect other body systems, nor was the growth rate different between treated and control males. Weights of the reproductive organs (testes, epididymides, seminal vesicles, and ventral prostate) were comparable between treated and control animals (Odum and Ashby, 2000).

In a study examining the effects of NP on sperm in mice, sperm capacitation (next to the last step in spermatozoa maturation) and the acrosome reaction were altered; these events are necessary for sperm to be capable of fertilization (Fraser et al., 2006). Cauda epididymal sperm were collected from mature mice, and then incubated in 100 nmol NP/L (22 μg/L). The production of cyclic adenosine monophosphate (cAMP) was significantly stimulated in NP-treated sperm compared with untreated control suspensions (Fraser et al., 2006). Numerous studies have demonstrated that cAMP plays a pivotal role in sperm physiology, with many treatments that accelerate sperm capacitation causing an increase in cAMP. In another study demonstrating NP effects on rats, male Wistar rats were orally given 1, 10, and 100 μg NP/kg/day for 45 days (Chitra et al., 2002). Consequently, the weights of the testes and epididymides significantly decreased, and epididymal sperm counts decreased in a dose-dependent manner while the

activities of antioxidant enzymes (superoxide dismutase, catalase and glutathione reductase) also significantly decreased (Chitra et al., 2002).

In a multi-generation study, Sprague-Dawley rats were exposed by diet to 200, 650 and 2000 parts per million (ppm) NP (which correspond to dietary intakes of 9-35, 30-100, and 100-350 mg/kg/day). The effect in males in the F_2 generation was a reduced epididymal sperm density (8 percent and 13 percent reduced in the 650 ppm and 2000 ppm treatment groups, respectively), and testicular spermatid count was reduced by 13 percent in the 2000 ppm treatment group (Chapin et al., 1999). In a study by Han et al., Sprague-Dawley rats were treated by gavage with 0, 125, and 250 mg NP/kg/day. Rats treated with 250 mg NP/kg/day had a decreased absolute weight of the epididymis, and sperm number in the head of the epididymis was also dramatically decreased (Han et al., 2004). Han et al. also demonstrated the level of testosterone significantly declined in the 250 mg NP/kg/day group, follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels in serum were higher in both NP treatment groups compared with the control group, and the histology within the seminiferous tubules was different within treatment groups. The 125 mg NP/kg/day group had less compact cells, and the 250 mg NP/kg/day group had irregular and disordered arrangement with shedding of cellular material from the seminiferous epithelium compared with the compact and regular arrangement of cells in the control group (Han et al., 2004). The high doses of NP used by Han et al. for a 50 day treatment period also resulted in increases of the relative weights of the kidney and liver of treated animals, which may suggest chronic toxicity.

Laurenzana et al. examined the effect of NP on serum testosterone levels and testicular steroidogenic enzyme activity. Male rats in the F_1 and F_2 generations were exposed through dams or directly through dietary doses of 0, 25, 200 and 750 ppm throughout gestation until sacrifice (PND 2, 50, or 140) (Laurenzana et al., 2002). At PND 2, serum testosterone levels were significantly decreased in the F_1 generation (Laurenzana et al., 2002). The activity of 17α–hydroxylase/C17, 20 lyase (P450c17) was significantly decreased in testicular microsomes of the F_2 generation on PND 50 and PND 140, and testicular β-nicotinamide adenine dinucleotide phosphate (NADPH) P450 reductase activity was also reduced at PND 50 and 140 in the F_1 and F_2 generations (Laurenzana et al., 2002). The activity of P450c17 and NADPH P450 reductase enzymes are necessary for testosterone synthesis. Results from Laurenzana et al. suggest NP can inhibit the activity of enzymes involved in testosterone synthesis.

Gong et al. examined the effect of NP on steroidogenesis in rat Leydig cells using both *in vivo* and *in vitro* exposures. Serum testosterone and LH levels were measured after males were treated with 1, 125, and 250 mg/kg/day for 50 days by gavage. Leydig cells were cultured for 48 hours in low concentrations (0 to 0.022 mg/L) and higher concentrations (0.11 to 5.5 mg/L) (Gong and Han, 2006). *In vivo* exposure to NP resulted in a dramatic decline in testosterone levels at the dose of 250 mg/kg/day, while the LH level increased at the 125 and 250 mg/kg/day dose. The response of Leydig cells to *in vitro* NP exposure was biphasic; at low NP concentrations there was an increase in testosterone levels, while at higher concentrations there was a decrease in testosterone levels (Gong and Han, 2006).

Females

The reproductive effects of NP on female laboratory rodents are less well-examined: endpoints examined include uterotrophic effects, and age at vaginal opening. Administration of increasing doses of NP (1.0, 2.0, and 4.0 mg) to immature female Sprague-Dawley rats 24 hours before

sacrifice resulted in significant increases in the following uterine parameters: weight, protein content, DNA content (in the 4.0 mg group only), and uterine peroxidase activity (Lee and Lee, 1996). Uterine peroxidase activity is known to be sensitive to modulation by substances which modify the uterine responses to estrogen (King et al., 1981). Pre-pubertal Long Evans rats given oral doses of NP (50-100 mg/kg) on PND 21 to 35 had a significant increase in uterine weight, and the age at vaginal opening of treated rats was younger compared with the controls (Laws et al., 2000). Oral exposure to 50, 100, and 200 mg NP/kg resulted in a significant increase in uterine weight compared with exposure to 50, 100, and 200 mg NP/kg by subcutaneous injection (Laws et al., 2000).

In a multi-generation study conducted by Chapin et al., Sprague-Dawley rats were exposed by diet to 200, 650 and 2000 ppm NP. Uterine weight at PND 21 was increased in F_1 females treated with 650 and 2000 ppm NP. Vaginal opening was accelerated by approximately 2 days in the 650 ppm exposure group, and by approximately 6 days in the 2000 ppm exposure group (Chapin et al., 1999).

In Vitro Human Cell-Line Study

Males & Females

The reproductive effects of NP on humans are not well known. Ohshima et al. examined the effects of NP on the enzyme 11 β-hydroxysteroid dehydrogenase (11 β-HSD type 1) using an *in vitro* approach. The 11 β-HSD type 1 enzyme regulates the bioavailability of glucocorticoids by inter-converting physiologically active glucocorticoids to their inactive metabolites. Human liver microsomal 11 β-HSD type 1 and type 2 activities were inhibited by NP (Ohshima et al., 2005). Ohshima et al. also assessed the gonads and accessory genital glands. Expression of an 11 β-HSD isozyme in a reproductive organ suggests the organ may be adversely affected by NP exposure. The porcine uterus is known to express 11 β-HSD type 1 (Yang et al., 1996). The human testis, ovary, and prostate expressed 11 β-HSD type 1 (Ohshima et al., 2005). Except for the prostate, only small amounts of the 11 β-HSD type 2 isozyme were detected in these human tissues compared to kidney (Ohshima et al., 2005).

Summary

Overall, the exposure of males and females to NP results in effects consistent with estrogenic activity of NP. Testicular tube diameter and uteri primarily exhibit seemingly minor alterations in size and weight. Alterations in reproductive organ weight do not necessarily indicate toxicity, but indicate estrogenic activity by NP. The hormonal profiles of male laboratory animals showed reductions in testosterone, increases in FSH, and increases in LH. In females, the earlier average age of vaginal opening suggests puberty occurred at a younger age.

Developmental Toxicity

Introduction

Developmental toxicity is generally considered to include any adverse effects induced by exposure to a toxic chemical during the developmental period (e.g., *in utero*, *in ovo*, during larval development, or postnatally until sexual maturation). Exposure of the parents prior to conception can also contribute to developmental toxicity. Adverse developmental effects can be manifested at any point in the life span of the organism. Developmental toxicity studies on

laboratory animals and humans may provide data that are helpful for deducing the mechanism by which NP can alter development in marine organisms.

Laboratory Rodent Studies

Generally, maternal exposure to hormonally active substances during pregnancy (particularly the period of sexual differentiation) produces adverse effect(s) in the reproductive organs of offspring. Pregnant Long Evans rats gavaged with 100 mg NP/kg body weight on gestation days 15 to 19 had female offspring with advanced lobular development of their mammary gland on PND 22, an increase in uterine weight compared with controls, and a markedly lower staining intensity of progesterone receptors in the mammary gland epithelium (Moon et al., 2007). In contrast, pregnant Donyru rats gavaged with 0, 0.1, 10, or 100 mg NP/kg daily from gestation day 2 up to the day before weaning (PND 21) produced female offspring who had no significant effects on the reproductive system. Exposure of rats to 0.1 – 100 mg NP/kg did not affect uterine growth and development, vaginal opening, hormonal secretion, estrous cyclicity, and morphology of the reproductive organs compared with controls (Yoshida et al., 2003).

A study by Kimura et al. examined the effect of gestational exposure to NP on the development and fertility of female and male ICR mouse offspring. On day 5 to 20 of gestation, pregnant ICR mice were dosed with subcutaneous injections of 1/1000, 1/100 and 1/10 the LD_{50} of NP (1231 mg/kg) (Kimura et al., 2006). There were no significant differences in litter size, sex ratio, or gestational length. Treatment with 1/100 the LD_{50} of NP significantly increased ovarian weight of the offspring, and the uterine weights tended to increase in a dose-dependent manner with large variations (Kimura et al., 2006). The absolute testis weight of males was dose-dependently reduced by gestational exposure to NP; there were no significant differences in testis weights relative to body weight (Kimura et al., 2006). The weights of the testis and epididymis of the 1/10 the LD_{50} of NP group were the most significantly reduced (Kimura et al., 2006). In another study, prenatal exposure of Wistar rats to 75 mg NP/kg body weight from gestational day 11 to 18 resulted in no differences on PND 11 in testis weight, histopathology, or length or diameter of the seminiferous tubules compared with the control group (Dalgaard et al., 2002). The number of Sertoli cells was also comparable between NP-treated and control rats (Dalgaard et al., 2002).

Prenatal exposure of laboratory rodents to NP also results in neurobiological alterations, including some sexually dimorphic behaviors. Masculinization of the sexually dimorphic nucleus of the medial preoptic area (SDN-POA) of the brain depends on perinatal estrogenic exposure during a critical period. Pregnant rats and their pups were continuously exposed to NP through their feed from gestational day 7 until sacrifice at PND 50 (Scallet et al., 1999). NP-treated males had a smaller SDN-POA on average compared with controls (Scallet et al., 1999). The SDN-POA of females were unaltered (Scallet et al., 2001).

Pregnant Sprague-Dawley rats and their offspring were fed diets containing 0, 25, 500, or 2000 ppm NP beginning on gestational day 7 (offspring continued on the same maternal diet until PND 77), and the offspring were evaluated for behavioral alterations. A significant effect of sex and intake of flavored solutions was noted in the offspring (Ferguson et al., 2000). Females consumed nearly twice as much regular water as males over a 3-day period, and females consumed approximately 1.5 times the amount of salt solution as males from the same treatment groups (with the exception of the offspring from the 2000 ppm treatment group) on PND 73 to PND 75 (Ferguson et al., 2000). Pregnant F344/N rats orally dosed with 0.1, or 10 mg NP/kg/day beginning on gestation day 3 until PND 20 had male offspring that displayed altered

behavior in a passive avoidance test (Negishi et al., 2004). NP-treated male offspring tended to delay entry into a dark compartment, and exhibit slightly fewer avoidance responses compared with controls (Negishi et al., 2004).

Exposure to NP also affects synaptogenesis in primary cultures of fetal hypothalamic cells. Fetal rat hypothalamic cells were cultured in 1, 10, 100, and 1000 nM NP; the synaptic density (synapsin 1-positive area / MAP 2-positive area) was significantly increased by 10 nM NP treatment and decreased by 100 nM and 1 µM NP treatments (Ohtani-Kaneko et al., 2002).

Avian Studies

The effects of NP have been examined in the development of immune and endocrine organs of Japanese quail embryos. Japanese quail embryos were injected with a volume of 20 µL containing 1, 10, or 100 µg NP/g egg. Injection of NP into embryonated yolks increased the disappearance of the lymphoid cells from the lymphoid of the bursa at 10 µg NP/g egg, decreased the height of the simple cuboidal epithelial cells surrounding the thyroid follicle at 100 µg NP/g egg, and increased the follicle-like structure in the thymus on the male embryo at 100 µg NP/g egg (Razia et al., 2006).

Human Studies

Disturbances in hormonal regulation during fetal or postnatal development in humans may induce adverse effects on the reproductive system of male and female offspring. There are numerous estrogen responsive tissues which could be affected by exposure to NP such as the testes in males, and mammary glands and placentas in females. Estrogens play an important role in regulating functional differentiation of the placental villous trophoblast. In a longitudinal study of fetal exposures to endocrine disrupting chemicals in Japan, NP was detected in umbilical cords, and evidence showed puberty in prenatally exposed boys and girls occurred at an earlier age (Mori, 2000). The effect of NP on human placental development was also examined using an *in vitro* model of chorionic villous explants. Estrogen receptor (ER) expression was unaffected, but hormone and cytokine secretion were significantly modulated. A gradual increase of β human chorionic gonadotropin (hCG) and a decrease in migration inhibitory factor (MIF) production was observed in NP-treated versus control cultures (Bechi et al., 2006b). Similarly, treatment of placental explant cultures with NP significantly increased β-hCG secretion and trophoblast cell apoptosis, but did not modify ER expression (Bechi et al., 2006a).

Summary

The effects of NP on development in laboratory animals, Japanese quail, and humans are less conclusive compared with reproductive effects. Prenatal exposure to NP appears to have effects consistent with those of other estrogenic compounds (e.g., bisphenol-A) such as early mammary gland development in female offspring. Neurobiological alterations—such as sexually dimorphic behaviors—were also noted as a result of exposure to NP.

Cancer

Introduction

While there are no NP lifetime carcinogenicity assays using rats or mice, there are studies showing that exposure to NP causes effects that have been associated with cancer.

Laboratory Rodent Studies

F344 rats were given NP in the diet at concentrations of 25 or 250 ppm for 28 weeks. The exposed rats had a higher incidence of adenomas and carcinomas, combined, than did rats given a diet without NP. DNA from lung tissue of rats given 25 or 250 ppm NP had an increased amount of 8-hydroxy-2-deoxyguanosine, suggesting the formation of reactive oxygen species during metabolism of NP.

In a 28-day toxicity test in Sprague-Dawley rats, animals given a daily dose of NP (250 mg per kilogram body weight) by oral gavage had enlarged livers and kidneys (Woo et al., 2007). Histological examination of livers of immature male Sprague-Dawley rats given 60 mg/kg body weight NP by i.p. injection found increased mitotic index and abnormal mitoses (Zumbado et al., 2002).

Effects on Cultured Cells

At concentrations of 5 mg/L or 10 mg/L NP, increased the DNA content of cultures of 3T3-L1 cells by 32 percent or 68 percent, respectively, above the DNA content of cells cultured in the absence of NP (Masuno et al., 2003). In a 2-stage initiation-promotion transformation assay using BALB/3T3 cells, NP acted as a promoter (Sakai, 2001).

HeLa cells cultured in the presence of NP had more breaks in DNA than did cells cultured without NP (Park and Choi, 2007). At a concentration of 10 µmoles/L (2.2 mg/L), NP killed 55 percent of cultured MG63 human osteosarcoma cells (Wang et al., 2005). Jumket cells cultured for 24 hours in the presence of 10 µmoles/L NP, were killed.

Summary

There is very little information on the carcinogenicity of NP in the literature. The information available gives some reason for concern, but significantly more information is needed for a determination on the carcinogenicity of NP.

Obesity

Introduction

Stemp-Morlock (2007) observed that the obesity rate has greatly increased over the past 20 years. An estimated one-third of U.S. adults are overweight and more than one-third of U.S. children are overweight or at risk for becoming overweight. There is a strong association between obesity and a number of health issues such as diabetes, coronary heart disease, hypertension, and gall bladder disease. Traditionally obesity has been viewed as a result of reduced physical activities and increased caloric intake. Data from recent studies, however, suggest that exposure to chemicals that perturb the critical pathways in lipid formation, lipid metabolism or energy balance could also initiate or exacerbate obesity. While data are limited, they seem to suggest that NP may possess certain obesogenic properties.

Laboratory Studies

The study of environmental obesogen is an outgrowth of endocrine disruptor research. Hormones are key players in the development and maintenance of adipose tissues. In adults, sex steroids together with growth hormone have fat mobilizing properties (anti-adipogenic), whereas cortisol and insulin have lipogenic effects (Grun and Blumberg, 2007). Adachi et al. (2005)

investigated and found that NP promoted insulin secretion in rat pancreatic islets. NP was shown to bind estrogen receptors (ERα and ERβ) (Shelby et al., 1996; Waller et al., 1996) and these receptors were expressed in rat pancreatic islets (Adachi et al., 2005). ICI 182780 (ICI), an estrogen receptor blocker, suppressed the increase in insulin secretion in NP stimulated pancreatic islets. This led Adachi et al. to conclude that estrogen receptor binding by NP is required for an increase of insulin secretion. The authors also pointed out that the insulin inducing effect could potentially cause hyperinsulinemia, resulting in obesity, exhaustion of pancreatic β-cells, and diabetes.

Treatment with NP significantly stimulated the accumulation of triacylglycerol in mature adipocytes differentiated from 3T3-L1 preadipocytes (Wada et al., 2007). The lipid accumulation was time- and dose-dependent. Increased adipocyte size was noted. Upregulation of expression of genes involved in lipid metabolism, adipocyte differentiation, and inflammation were also observed. Specifically, an increase in the levels of phospholipase A_2 and phospholipase C that are involved in lipid metabolism and TNF-α, an adipocytokine involved in insulin resistance, were seen.

Masuno et al. (2005; 2003), on the other hand, provided data to indicate that NP caused cell proliferation but not lipid accumulation in differentiated 3T3-L1 cells. The presence of NP in the cell cultures caused a dose-dependent increase in DNA contents. Use of bromodeoxyuridine to label DNA during synthesis confirmed that NP enhanced 3T3-L1 cell proliferation. However, triglyceride levels and lipoprotein lipase activity were down, suggesting that NP may interfere with terminal differentiation of adipocytes. ICI suppressed NP stimulated cell proliferation but had no effect on NP induced reduction in lipid accumulation. Using ICI, Masuno demonstrated that the NP stimulated cell proliferation was mediated by the estrogen receptor; whereas NP's interference with terminal differentiation was mediated by a mechanism other than the estrogen receptor.

Summary

Existing data do not provide conclusive evidence that NP is an environmental obesogen. However, several laboratory models suggest that NP may possess obesogenic properties.

Thyroid

Introduction

Thyroid hormones, thyroxine (T4) and triiodothyronine (T3), have diverse functions. They are essential to brain development, influence growth via stimulation of growth hormone, and regulate basal metabolic rates, as well as lipid and carbohydrate metabolism (Greenspan and Gardner, 2003). Environmental chemicals can disrupt thyroid hormone (TH) functions by preventing the biosynthesis via the inhibition of iodide uptake or thyroid peroxidase activity, interfering with the activity of transthyretin that transports THs to target tissues, increasing the metabolism via deiodinases and uridine diphosphate glucuronyltransferase enzymes, or perturbing the binding to thyroid hormone receptors (Zoeller, 2007). The resulting hypothyroidism or thyroid hormone dysregulations in adults may lead to fatigue, weight gain, weak pulse, cold intolerance, mental sluggishness, and depression. Such dysregulation during the perinatal period, on the other hand, could cause cretinism in the affected person, which is characterized by having a short stature, poor motor skills, moderate to severe mental retardation.

Laboratory Studies

Ishihara et al. (2003) investigated the effect of nonylphenol (NP) on 3,3',5-L-$^{[125I]}$triiodothyronine ($^{[125I]}$T3) binding to purified Japanese quail transthyretin (qTTR), a major thyroid hormone-binding protein in plasma, and to the ligand-binding domain of thyroid hormone receptor beta (qTR LBD). The study revealed two classes of binding sites to qTTR, with binding constant (Kd) values of 6.9 and 185 nM, and a single class of binding sites to qTR LBD, with Kd value of 0.31 nM. NP was effective in completely inhibiting $^{[125I]}$T3 binding to qTTR at higher concentrations. Its potency, however, was two orders of magnitude less than that of T3. NP had an insignificant effect on $^{[125I]}$T3 binding to qTR LBD. These results show that NP targets qTTR rather than qTR LBD.

The pituitary is a target of THs. The endocrine disrupting potential of NP was determined by its effect on the cell proliferation of TH-dependent rat pituitary GH3 cell line (Ghisari and Bonefeld-Jorgensen, 2005). NP elicited an inhibitory effect on cell growth. The authors concluded that NP has the potential to exert TH disruption via the pituitary to increase the risk of a negative impact on fetal brain development. However, in a study on the effect of NP on TH-dependent dendritic development of Purkinje cells in mouse cerebellar cultures using serum-free defined medium, unlike bisphenol A, NP did not induce any inhibition, but significantly promoted the dendritic extension of Purkinje cells in the absence of THs (Kimura-Kuroda et al., 2007).

Okada et al. (2005) previously demonstrated that protein disulfide isomerase (PDI) was a target molecule of bisphenol A. In the current study the investigators extended the testing to NP. PDI plays a key role in protein folding as an isomerase and also possesses a $^{[125I]}$T3-binding activity. NP was shown to possess inhibitory effects on the isomerase activity of PDI. The result suggests that NP not only has inhibitory effects on the isomerase activities of PDI but also infers that NP may compete with T3 for receptor binding.

A 28-day repeated oral dose toxicity study of NP was performed for an international validation of the "Enhanced Organisation for Economic Co-operation and Development Test Guideline 407" paying particular attention to the sensitivity of individual endocrine-related parameters (Woo et al., 2007). Sprague-Dawley rats, each group consisting of ten males and ten females, were administered NP once daily by gavage at doses of 0 (control), 10, 50, or 250 mg/kg body weight. An increase of thyroid weight in males was detected from the 50 mg/kg exposure. However, in a developmental study, an injection of NP into Japanese quail embryos decreased the height of simple cuboidal epithelial cells surrounding the thyroid follicle (Razia et al., 2006), which is inconsistent with the previous finding.

To assess interference with endocrine regulation of the thyroid axis, rats (female, ovariectomised) were treated for 12 weeks with NP and other endocrine disruptors on the background of a soy-free or soy-containing diet, and endpoints relevant for regulation via the thyroid axis were measured (Schmutzler et al., 2004). NP inhibited thyroid peroxidase, but increased levels of T4 in rats on a high soy diet and T3 in rats on a low soy diet. There appears to be no uniform, obvious pattern in the effects, but NP elicited a spectrum of alterations, arguing for multiple targets of interference with the complex network of thyroid hormone action and metabolism.

Summary

The available data demonstrate that NP can potentially interfere with TH functions. NP was shown to interfere with TH biosysnthesis, transport, and receptor binding. While it could be inferred that the disruption of TH functions would adversely impact brain development or skeletal growth, additional scientific evidence will be required to establish such connections.

Immune System

Introduction

The immune system is our main defense mechanism against invading microorganisms or tumor growth. Suppressing the immune system would weaken our defense capabilities. Overstimulation of the immune system during an infection, however, could cause extensive collateral damages—"spill-over" destruction of surrounding but otherwise healthy tissues that may prove fatal in some instances. Dysregulation of the immune system in other situations may lead to autoimmunity—attacking "one's own tissues without cause or provocation."

The immune system is under tight, complex regulation to ensure that it continues to function at the optimal range. Existing data suggest that NP could perturb this regulatory apparatus, leading to weakened defense capabilities or detrimental overstimulation of immune functions as an end result. It appears that NP can also affect the immune system indirectly via the neuroendocrine system. Thyroid and sex hormone systems are immunoregulators (Berczi, 1997), and it should not come as a surprise that NP, which is known to disrupt thyroid and estrogen functions, can potentially impact the immune system.

Laboratory Animal Studies

It is interesting to observe that NP seems to have stimulatory effect on thymus cells *in vivo*. Yamashita et al. (2003) reported that NP at 10^{-5} M (2.2 mg/L) in drinking water given to mice for 6 weeks did not affect the body weight of the mice, spleen and thymus cell numbers, and serum immunogloburin concentration. The proliferative responses of spleen cells cultured *in vitro* were not changed. However, the proliferative responses of thymus cells from NP-treated mice were enhanced.

Razia et al. (2006; 2005) studied the effects of NP on the immune organs of Japanese quail and its embryos. In the 2005 study, birds were injected with NP in doses of 1000, 100 and 10 ng/g body weight from 4 to 7 weeks of age. Injection of NP tended to induce many empty vacuoles and increased connective tissue in the bursa of Fabricius (part of the immune system in birds) but did not affect the structures of the spleen and thymus. In the 2006 study, NP was injected into the yolk of embryonated eggs. Injection of NP increased the disappearance of lymphoid cells from the lymphoid follicles in the bursa.

Lee et al. (2003) investigated the influence of NP on allergic immune responses. In this study, they examined the effects of NP on production of IL-4, a cytokine closely associated with allergic immune responses. Their findings indicate the possible enhancement of allergic response by NP through increasing IL-4 production in CD4+ T cells and antigen-specific IgE levels in the sera via the stimulation of Ca2+/calcineurin-dependent NF-AT activation.

In a two-generation feeding study, Karrow et al. (2004) evaluated the potential for NP to modulate certain immune parameters. Pregnant female Sprague-Dawley rats were exposed to NP (0, 25, 500, and 2000 ppm) in their feed for 65 days, beginning 7 days into gestation. The

F(1) generation male and female offspring were exposed *in utero* at the respective treatment levels, commencing the 7th day of gestation, and continuing through to 64 days of age. Changes in splenic antibody-forming cell response, natural killer cell activity, and leukocyte numbers were used to evaluate NP immunotoxicity. The results from this study indicate that dietary exposure to NP can increase splenic natural killer cell activity and splenocyte subpopulation numbers in the F(1) generation rats, without similar changes to the F(0) generation. This suggests that the *in utero* period is a sensitive window for NP exposure.

In Vitro Studies

To elucidate relevance of estrogen distruption to immune responses, Sakazaki et al. (2002) investigated whether ERα exists in mouse splenic B cells and T cells, and the effect of 17 beta-estradiol and endocrine disrupting chemicals such as NP had on lymphocyte mitogenesis. ERα was identified in both male and female mouse splenic cells. Crude splenic cells were stained with anti-ER antibody, and the distribution of ERα in the splenic B cells and part of the splenic T cells was confirmed by flow cytometry. 17 beta-Estradiol inhibited B cell mitogenesis at the concentration of 10^{-8} M (2.2 µg/L) and T cell mitogenesis at the concentration of 10^{-6} M (220 µg/L). NP suppressed lymphocyte mitogenesis at the concentration of 10^{-6}-10^{-5} M. The authors concluded NP may suppress lymphocyte mitogenesis through ERα in B and T cells.

Yao et al. (2005; 2006) investigated the cytotoxicity of NP. In the 2005 study, the effects *in vitro* of NP on apoptosis (the process of programmed cell death) in rat thymocytes were investigated. Thymocytes were treated with NP at 0.1, 1, and 10 ppm, respectively. The results showed that NP induced apoptotic death in thymocytes. These findings suggest that NP may induce apoptosis so as to affect the immune system function. In the followup 2006 study, Yao et al. showed that the cytotoxic effects of NP involved DNA fragmentation (DNA ladder), characteristic of apoptosis. Staining of NP-treated thymocytes showed the typical apoptotic nuclei condensation and fragmentation of chromatin. The rates of apoptosis of the NP-treated thymocytes increased significantly at 4 and 6 hours.

Several studies were conducted to investigate the effects on NP on compromising macrophage functions. Yoshitake et al. (2008) demonstrated that NP suppressed nitric oxide (NO) production and NF-kappaB activation in lipopolysaccharide (LPS)-stimulated macrophages through an estrogen receptor (ER)-dependent pathway. Yoshitake et al. investigated these effects in a mouse macrophage cell line. The results revealed that NP dose-dependently suppressed LPS-induced NO production, as reflected by decreased NO(x) content. The suppressive effects of NP were blocked by the ER inhibitor, ICI. You et al. (2002) studied the effects of NP on the production of NO and tumor necrosis factor-alpha (TNF-alpha), and on the level of inducible NO synthetase and TNF-alpha gene expression in mouse macrophages. NP alone did not affect NO or TNF-alpha production. In contrast, NP inhibited LPS-induced NO and TNF-alpha production in a dose-dependent manner. Treatment with ICI, an estrogen-receptor antagonist, inhibited the suppressive effects of NP. These results demonstrate that NP may affect the regulation of the immune system function by reducing NO and TNF-alpha production through the ER receptor. In a similar study, Hong et al. (2004) investigated the effect of NP on mouse macrophage production of TNF-alpha and NO in response to bacterial endotoxin *in vitro*. NP was shown to inhibit LPS-induced NO production. Two subsequent experiments suggest that NP effects on TNF-alpha and NO in macrophages are a result of down-regulation of gene transcriptions. The activation of the transcription factor NF-kappaB (Igarashi et al., 2006) and IFN-β promoter (Ohnishi et al., 2008) are essential for the production of TNF-alpha and NO. Igarashi et al. and

Ohnishi et al. demonstrated that NP inhibited LPS-induced activation of NF-kappaB and IFN-β promoter.

Summary

NP appears to possess complex immuno-modulating effects. It could stimulate or suppress the immune system. It could also alter immune response pathways. NP's immunosuppressive effects could potentially compromise our abilities to fight infections. It is more difficult to interpret NP's immune-stimulative effects. Existing data do not provide conclusive evidence that such stimulatory effects could predispose the affected individuals to autoimmunity or allergy.

Nervous System

Introduction

NP has both indirect and direct effects on the nevous system. Since gonadal hormones in conjunction with other neurotrophins regulate cell death, neuronal migration neurogenesis, and neurotransmitter plasticity, NP, in disrupting sex hormone functions, can affect brain development. In disrupting thyroid functions, NP can also affect the development of the nervous system because thyroid hormones play an important role in prenatal and neonatal development of the brain (Porterfield and Hendrich, 1993). Early hypothyroidism, for example, caused stunted dentritic growth in hippocampal Cornu Ammonis zone 3 neurons, resulting in cognitive effects including impaired memory, spatial perception, and attention problems (Schantz and Widholm, 2001). In addition, NP may directly cause neurodegeneration. Experimental data from literature indicate that NP has a significant impact on the dopaminergic system.

In Vitro Studies

NP may directly cause neurodegeneration. The treatment of neural stem cells (NSCs) with NP for 24 hours inhibited cell growth in a concentration-dependent manner (Kudo et al., 2004). In addition, treatment with NP resulted in nuclear condensation and DNA fragmentation (morphological changes due to apoptosis) in NSCs after 12 hours of exposure. Furthermore, an exposure to NP led to the accumulation of cells at a specific point of the cell cycle and a reduction in levels of major regulatory proteins that allow the cell to continue to move through the cycle. Together, these results indicate that NP may exhibit a potent cytotoxicity through apoptosis and suggest that NP may affect neurogenesis in the central nervous system. In another study, Kim et al. (2006) used undifferentiated human embryonic stem (hES) cells and the neural progenitor cells derived from them to investigate the potential toxicity of NP. The results showed that the cytotoxic effects of NP involved DNA fragmentation. The NP-induced apoptosis was concomitant effects seen in other studies. In addition, the investigators observed that hES cell-derived neural progenitor cells had a higher sensitivity to the toxicants than undifferentiated hES cells.

The data provided by Bevan et al. (2006) suggest that NP may elicit very disparate effects along divergent signaling pathways than those that arise from the actions of physiological levels of endogenous estrogens. The data highlight important implications with respect to potentially deleterious effects of NP exposure during early neural development. Treatment of dissociated embryonic *Xenopus* spinal cord neurons with NP did not alter cell survival but inhibited neurotrophin nerve growth factor (NGF)-induced neurite outgrowth. These effects were also

seen with comparable concentrations of 17 beta-estradiol (E2). Effects of NP were not inhibited by the nuclear ER antagonist ICI, but were inhibited by the G-protein antagonist, pertussis toxin. These data suggest that the effects of NP are ER independent but G-protein dependent. The ability of NP to inhibit NGF-induced neurite outgrowth without altering survival was also seen in a rat pheochromocytoma cell line. As with Xenopus neurons, the inhibitory actions of NP in pheochromocytoma cells were not antagonized by ICI. In another study, Khan et al. (2003) investigated the influence of alkylphenol endocrine disrupters and the synthetic estrogen diethylstilbestrol on inositol-1,4,5-trisphosphate (IP(3))-sensitive Ca(2+) channels from porcine cerebellum and rat testicular membranes. All alkylphenols and diethylstilbestrol inhibited the extent of IP(3)-induced Ca(2+) release from both cerebellar and testicular microsomes. NP was the most potent compound tested. These results illustrate another mechanism by which NP can disrupt endocrine function without the involvement of estrogen receptors.

Dopaminergic System

Other experimental data from literature indicate that NP has an adverse impact on the dopaminergic system. The following is a synopsis of relevant background and data.

The phenotypic expression of behaviors is the outcome of interacting cortical neuronal networks that are modulated by subcortical components such as the cholinergic neurons of Myenert's basal nucleus, dopaminergic neurons of the Ventral Tegmental Area (VTA), serotongeric neurons in the Raphe nuclei, norepinephrine neurons in the Locus Coeruleus, and histamine neurons in the posterior hypothalamus (Viggiano et al., 2003). Though behaviors emerge from complex interactions, the dopamine systems are very important for the phenotypic expression of attention and reward. It is recognized that the mesolimbic VTA and the nigrostriatal dopaminergic sytems are essential to reward-based learning, novelty-induced behavior, attention, and activity (Andersen and Teicher, 2000; Berridge and Robinson, 1998; Carlsson, 1993). The dysfunction of dopaminergic systems has been associated with neuropsychiatric disorders such as Parkinson's disease, schizophrenia, attention-deficit hyperactivity disorder (ADHD), and autism. Certain drugs used to treat schizophrenia and ADHD, for example, target the dopamine system. Most of the anti-psychotic medications for schizophrenia are dopamine receptor antagonists, whereas drugs for treating ADHD are usually psycho-stimulants that modify dopamine transmission (Viggiano et al., 2003). Methylphenidate, which blocks dopamine re-uptake and effectively increases the synaptic concentration of dopamine, has been used to treat ADHD (Medscape, 2006). Addictive drugs such as cocaine and amphetamine, on the other hand, create a "reward" reinforced behavior by modifying the dopaminergic transmission of VTA.

Sex differences in striatal dopamine content or density of dopamine receptors (D_1 and D_2) during development suggest that sex steroid hormones may mediate the development of dopamine systems in the brain (Andersen and Teicher, 2000; Ferretti et al., 1992). In adults, estrogen appears to be neuroprotective (Marx and Lieberman, 1998). Prenatal "excess" exposure to estrogen seems to have an opposite effect than in adulthood. That evidence was seen in psychotic patients prenatally exposed to diethylstilbestrol (Katz et al., 1987). On the other hand, Turner syndrome, in which a missing X chromosome that causes an absence of estrogen during perinatal life, is associated with cognitive problems and psychosis (Bamrah and Mackay, 1989).

While the development of the dopamine systems is influenced by sex hormones and disruption of sex hormone functions can impact this system, some data, as discussed, suggest that NP could affect the nervous system including the dopamine pathways via other mechanisms rather than via

estrogen disruption. The following data demonstrates NP's effects on the dopamine systems without defining whether these effects are related to endocrine disruption. NP was shown to increase hydroxyl radical formation in the rat striatum (Obata and Kubota, 2000), and enhance hydroxyl radical formation induced by 1-methyl-4-phenylpyridinium ion (Obata, 2006), known to cause neurodegeneration of the substantia nigra and produce acute Parkinsonian symptoms. Antioxidants, histidine and imidaprilat, on the other hand, were shown to suppress NP and 1-methyl-4-phenylpyridinium ion-induced hydroxyl radical generation in rat striatum (Obata, 2006; Obata et al., 2001).

Several studies investigated the causative relationship of NP and motor hyperactivity because of the observed hyperactivity in patients with pervasive developmental disorders, such as autism and ADHD. Masuo et al. (2004a) studied the effects of intracisternal administration of NP and other endocrine disruptors on spontaneous motor activity in neonatal rats. Treatment with NP caused significant hyperactivity during both dark and light phases in rats aged 4-5 weeks. In another experiment, Masuo et al. (2004b) also showed that intracisternal injection of NP in rats on postnatal day 5 caused an increase in spontaneous motor activities at 4 weeks of age. At the same time Masuo et al. observed that NP caused a deficit in dopamine neurons. Added to the evidence, Ishido et al. (2005) reported a single intracisternal administration of NP into 5-day-old male Wistar rats caused significant hyperactivity at 4-5 weeks of age. It was about 1.3- to 1.6-fold more active in the nocturnal phase than control rats. The gene expression of dopamine receptor D_{1A} was decreased by NP by 0.23- to 0.4-fold, whereas that of dopamine D_2 was increased by NP by 2- to 2.8-fold. The results suggest that neonatal treatment with NP can generate an animal model of ADHD, in which clinical symptoms are pervasive.

In a study to examine the relationship between NP and monoaminergic associated behavioral alterations, Negishi et al.(2004) exposed F344 rats perinatally to NP [0.1 mg/kg/day (low dose) and 10 mg/kg/day (high dose) orally] daily from gestational day 3 to postnatal day 20. NP exposure did not affect behavioral characteristics in an open-field test (8 weeks of age), in a measurement of spontaneous motor activity (12 weeks of age), or in an elevated plus-maze test (14 weeks of age). A passive avoidance test (13 weeks of age) showed that NP-treated offspring tended to delay entry into a dark compartment. An active avoidance test at 15 weeks of age revealed that low-dose NP-treated offspring exhibited slightly fewer avoidance responses. In a monoamine-disruption test using 5 mg/kg (intraperitoneal) tranylcypromine, a monoamine oxidase inhibitor, low-dose NP-treated offspring at 22-24 weeks of age failed to show a significant increment in locomotion in response to tranylcypromine, whereas control and high-dose NP-treated offspring significantly increased locomotion behavior after tranylcypromine injection. The results indicate that perinatal NP exposure irreversibly influenced the reception of fear-provoking stimuli (e.g., electrical shock), as well as monoaminergic neural pathways.

Summary

Studies conducted with cultured cells and tissues suggest that NP could adversely affect brain development and may cause neurodegeneration. Laboratory animal data also suggest that NP can specifically affect the dopamine system. Since the dysfunction of dopaminergic systems has been associated with neuropsychiatric disorders such as attention deficit/hyperactivity and autism, the concern is that NP may be a factor in the pathogenesis of such disorders.

Conclusions

Findings

This toxicological profile on NP describes its effects on freshwater and marine life, humans, and laboratory animals.

- NP is toxic to a wide variety of marine and freshwater vertebrate and invertebrate species in laboratory settings. Toxic effects include reproductive and endocrine effects as well as general and systemic toxicity.
- Most NP effects are associated with water concentrations ranging from 1 to 1000 µg/L, but there are some reports of effects at environmental concentrations less than 1 µg/L. Since most environmental concentrations are less than 1 µg/L, it appears that only the most vulnerable species are likely to be affected and only at the upper range of environmental concentrations. Although extrapolating the results of laboratory studies to environmental settings is common practice, it would be preferable to have data based on free-living marine organisms. Unfortunately, there is a paucity of these more-difficult studies.
- Most environmental concentration data are from fresh water systems. It would be useful to gather data on levels in marine environments, especially near municipal and industrial outfalls, landfills, and other possible point sources of NP.
- The exposure of males and females rodents to NP results in effects consistent with estrogenic activity of NP. Testicular tube diameter and uteri primarily exhibit seemingly minor alterations in size and weight. Alterations in reproductive organ weight do not necessarily indicate toxicity, but indicate estrogenic activity by NP.
- The effects of NP on development in laboratory animals, Japanese quail, and humans are less conclusive compared with reproductive effects. Prenatal exposure to NP appears to have effects consistent with those of other estrogenic compounds. Neurobiological alterations – such as sexually dimorphic behaviors – were also noted as a result of exposure to NP.
- There is very little information on the carcinogenicity of NP in the literature. The information available gives some reason for concern, but significantly more information is needed for a determination on the carcinogenicity of NP.
- The available data demonstrate that NP can potentially interfere with thyroid hormone functions, but more studies are needed before any determination can be made that this is an important effect of NP.
- NP appears to possess complex immuno-modulating effects. It may stimulate or suppress the immune system. Its immunosuppressive effects can potentially compromise an organism's abilities to fight infections. It is more difficult to interpret NP's immune-stimulative effects.
- Studies conducted with cultured cells and tissues suggest that NP could adversely affect brain development and may cause neurodegeneration. Laboratory animal data also suggest that NP can specifically affect the dopamine system. Since the dysfunction of

dopaminergic systems has been associated with neuropsychiatric disorders such as attention deficit/hyperactivity and autism, the concern is that NP may be a factor in the pathogenesis of such disorders.

Data Gaps

- Most of the environmental concentration data on NP are from fresh water systems; NP levels in the marine environment were not identified except around point and area sources.
- Little information on environmental fate and food chain exposure to NP was found.
- It is unknown how much plastics contribute to NP concentrations in the environment.
- Data on toxicity to marine organisms, especially free-living marine organisms, are lacking.
- Environment Exposure levels of human is unknown or at least not investigated in this report.
- Developmental toxicity of NP has not been well elucidated.
- Possible subtle effects in neurological development may occur, but is not well enough studied.

Recommendations

- Need to do more literature research and freshwater and ocean sampling of water columns and sediment to determine if there is a contamination problem.
- While reproductive and developmental effects in aquatic organisms are known to occur, other types of toxicity need further research.
- There is a need for further research on NP tissue levels in aquatic organisms for food chain exposure estimates.
- A review of the Ambient Water Quality Concentration levels developed by US EPA should be done to determine if they are still adequate. (AWQC published December 2005.)
- Research is needed to determine whether plastics are a significant contributor to NP environmental exposure.

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Appendix 1: Effects of nonylphenol on aquatic organisms

Species	Exposure (µg/L)	Effect	Reference
Reproductive Toxicity			
Flounder (*Psetta flesus)*	333	↑ vitellogenin (an egg yolk precursor protein expressed in female fish) levels	(Kirby et al., 2007)
Turbot *(Psetta maxima)*	29	↑ plasma vitellogenin ↑ zona radiata protein	(Larsen et al., 2006)
	29	↓ plasma, testicular, and biliary androstenedione & testosterone	(Labadie and Budzinski, 2006)
	30	↓ testosterone and β estradiol ↓ glucuronidation of testosterone and estradiol ↓ P450 aromatase	(Martin-Skilton et al., 2006)
Cod *(Gadus morhua)*	30	↓ glucuronidation of estradiol	(Martin-Skilton et al., 2006)
	29	↑ plasma vitellogenin	(Larsen et al., 2006)
Medaka *(Oryzias latipes)*	5.4	Hepatic vitellogenin levels (♂)	
	61	↓ Fecundity & fertility,	(Ishibashi et al., 2006)
	100 µmolar	↓ spermatozoa swimming speed ↓ ratio of motile spermatozoa	(Hara et al., 2007)
	30-100	↑ female/male ratio, mixed sex characteristics	(Balch and Metcalfe, 2006)
	25	induction of testis–ova and VTG	
	101	↓ reproduction	(Kang et al., 2003)
Rainbow trout *(Oncorhynchus mykiss).*	0.28 – 0.75	↓ embryo development & survival	(Lahnsteiner et al., 2005)
	0.13	↓ semen volume	
	1.05	↑ liver vitellogenin	(Ackermann et al., 2002)
	10.2	↑ liver zona radiate protein	
	220	Biochemical changes in liver	(Cakmak et al., 2006)
	6	↑ liver vitellogenin	(Thorpe et al., 2001)
	100	↑ liver vitellogenin	(Van den Belt et al., 2003)
	0.1 µM (22 µg/L)	↓ salmon gonadotropin releasing hormone2	(Vetillard and Bailhache, 2006)
Tilapia *(Oreochromis mossambicus)*	10-100 µM	↓ thymidine uptake in cartilage	(Ng et al., 2001)
Fathead minnow *(Pimephales promelas)*	5	EC50 vitellogenin induction	(Brian et al., 2005)
	10	vitellogenin induction	(Pickford et al., 2003)
	10	vitellogenin induction	(Marin and Matozzo, 2004)

Species	Exposure (µg/L)	Effect	Reference
Zebrafish *(Danio rerio)*	10 - 100	↑ Female/male ratio, ↓ swim-up	(Lin and Janz, 2006)
	17.7	↓ population growth	(Lin et al., 2005)
	39-100	Ovatestes @ 60 days, not after100-day recovery	(Hill and Janz, 2003)
	100	↓ ♂/♀ ratio ↓ % viable eggs, hatchability, and swim-up	
	500	vitellogenin induction	(Van den Belt et al., 2003)
	500	↓ ♀ Gonadosomatic index	(Yang et al., 2006)
	100	vitellogenin induction ♂	
	50	Thin F1 eggshells. ↓ Cat D & ↑ malformations in F2	
rare minnow *(Gobiocypris rarus)*	10	♂ liver lesions, ↑ VTG ↑ gonadosomatic index,	(Zha et al., 2007)
	30	Testis-ova	
Atlantic salmon *(Salmo salar)*	5-50	↓ brain p450 aromatase B mRNA ↑ zona radiata protein mRNA ↑liver & brain ER α, ↑VTG	(Meucci and Arukwe, 2006)
	15-50	↑ VTG in plasma & mucus	
	10-60	↑ VTG, ZR, ERα & ERβ mRNA	(Arukwe and Roe, 2008)
	15	↑ plasma VTG	(Meucci and Arukwe, 2005)
	≥5	↑ ZR protein in plasma & mucus	
Platyfish *(Xiphophorus maculates)*	80	Hypertrophied Sertoli cells & efferent duct cells. ↓cysts of spermatogenetic cells	(Kinnberg et al., 2000)
	14	↓ gonad development, ↓ spermiogenesis,	(Magliulo et al., 2002)
Swordtail *(Xiphophorus helleri)*	100	Testicular necrosis	(Kwak et al., 2001)
	0.2	↓ sword length	
Killifish *(Fundulus heteroclitus)*	65	↑ liver vitellogenin mRNA	(Garcia-Reyero et al., 2004)
	10 mg/kg	↑ liver vitellogenin	(Pait and Nelson, 2003)
Sheepshead minnow *(Cyprinodon variegates)*	5.6	Vitellogenin LOEL	(Hemmer et al., 2002)
	5.4	Vitellogenin LOEL	(Hemmer et al., 2001)
Worm *(Tubifex tubifex)*	610 µg/g sediment	LC_{LO}; Surviving adults had empty spermatheca if present, spermatic sacs had few germinal elements and no euspermatozons. Ovaries were present but ovocytes were not developed	(Bettinetti and Provini, 2002)
Pond snail *(Lymnaea stagnalis)*	105-124	↓ egg masses, ↑ embryo mortality, delayed development	(Lalah et al., 2007)

Species	Exposure (µg/L)	Effect	Reference
	100	↓ fecundity	(Czech et al., 2001)
Freshwater mudsnail *(Potamopyrgus antipodarum)*	10 µg/kg sediment	↑ number embryos	(Duft et al., 2003)
Zebra mussel *(Dreissena polymorpha)*	500	↑ vitellogenin	(Quinn et al., 2006)
Sea urchin *(Para-centrotus lividus)*	0.27	↓ fertilization (sperm toxicity EC50)	(Ghirardini et al., 2001)
Frog *(Rana pipiens)*	1-10	↑ intersex, ↓ males, ↑ gonadal development	(Mackenzie et al., 2003)
Daphnia magna	40	↓fecundity	(Brennan et al., 2006)
	25, 50	Altered sex ratio	(Zhang et al., 2003)
Midge *(Chironomus riparius)*	1	Altered sex ratio	(Lee and Choi, 2006)
Mysid shrimp	0.01	↑ VTG (not @ 1 or 100)	(Ghekiere et al., 2006)
Rotifer *(Brachionus calyciflorus)*	≥0.59µM (130 µg/L)	↓ population growth	(Radix et al., 2002)
General Toxicity			
Sea bream	200 µg/kg bw	↓ kidney Na+,K+-ATPase, ↑ plasma osmolality	(Carrera et al., 2007)
Turbot *(Psetta maxima)*	30	↑ micronuclei	(Barsiene et al., 2006)
	30	Borderline ↑ micronuclei	(Bolognesi et al., 2006)
Coho Salmon parr	≤ 2g/kg diet	No effect on smoltification	(Keen et al., 2005)
Rainbow Trout *(Oncorhynchus mykiss)*	1	vacuolation of epidermal mucous cells	(Burkhardt-Holm et al., 2000)
	10 (NOEC = 6.3)	↓ length & weight of fry	(USEPA, 2005)
Fathead minnow *(Pimphales promelas)*	14 (NOEC = 7.4)	↓ fry survival	(USEPA, 2005)
Shrimp *(Americamysis bahia)*	6.7 (NOEC = 3.9)	↓ length of offspring	(USEPA, 2005)
Medaka	61	Increased mortality	(Ishibashi et al., 2006)
Zebrafish	1 µM (220 µg/L)	CYP19A2 transcription	(Kazeto et al., 2004)
Platyfish *(Xiphophorus maculates)*	14	↑ mortality, ↓weight & length	(Magliulo et al., 2002)
Atlantic salmon (*Salmo salar*) smolts	10	↑ plasma cortisol	(Lerner et al., 2007b)
	100	Loss of osmoregulatory control	
	10	↓ gill (Na+,K+-ATPase) ↓ preference for & tolerance of seawater, 20% lower plasma insulin-like growth factor, ↑ mortality, 35% lower plasma T3	(Lerner et al., 2007a)
	20	↓ plasma IGF-I concentrations ↓ Smolt weights	(Arsenault et al., 2004)
Chinook salmon (*Oncorhynchus tshawytscha*)	20	↓Growth, ↓plasma IGF1	(Fernandez et al., 2007)

Species	Exposure (µg/L)	Effect	Reference
Japanese eel (*Anguilla japonica*) cultured testicular cells	≥10 µg/ml	proliferation of type A spermatogonia then developmental arrest enlargement of Sertoli cells	(Miura et al., 2005)
Chironomus riparius	5.65 µmol/L	LC50 water	(Maenpaa and Kukkonen, 2006)
	11.4 µmol/kg	LC50 body burden	
	2.04 µmol/kg	↓ survival, low FOC	
	9.23 µmol/kg	↓ survival high FOC	
	100-200 µg/g	Sediment tox ↓ weight ECLO	(Bettinetti et al., 2002)
	300-600 µg/g	Sediment tox LC50	
	484	24-hr LC10	(Lee et al., 2006)
	1	↑ Heat-shock protein mRNA	
	10 (marginal @ 1)	DNA strand breaks	
	10	↑ glutathione-S-transferase	
Chironomus tentans	1 - 100	↑ heat-shock protein & hemoglobin mRNA	(Lee and Choi, 2006)
Daphnia magna	140	48-hr EC50 mobility	(Brennan et al., 2006)
	130	48-hr EC50 moulting frequency	
	40	↑adult mortality	
	210 (250)	24-hr LC50 (NOEC)	(Milam et al., 2005)
	.46µM	↑ abnormal embryos	(LeBlanc et al., 2000)
Ceriodaphnia cornuta	20	48-hour LC50	(Hong and Li, 2007)
	10	48-hour LOAEL (mortality)	
Ceriodaphnia dubia	220	24-hr EC50	(Isidori et al., 2006)
	8	7-day EC50	
	200 (100)	24-hr LC50 (NOEC)	(Milam et al., 2005)
Copepod *(Eurytemora affinis)*	3	Delayed development	(Forget-Leray et al., 2005)
	15	↑ 10 day mortality	
Copepod *(Tigriopus japonicus)*	200	48-hr EC0 motility	(Marcial et al., 2003)
	0.1-10	Delayed completion of naupliar stages	
Aquatic mesocosm	29	↓ Cladocera, ↓ Copepoda ↑ rotatoria, some phytoplankton ↑, some ↓	(Hense et al., 2003)
Aquatic microcosm	~20	Changes in algal species composition and biomass	
bullfrog	234-936	Delayed tail resorption	(Christensen et al., 2005)
Sea urchin	0.94 - 18	Malformations	(Cakal Arslan and Parlak, 2007)
Clam *(Tapes philippinarum)*	25	↓Respiration rate ↓Absorption efficiency	(Matozzo et al., 2004)
	25	↓Superoxide dismutase	
	100	↓Re-burrowing	
Mussel *(Mytilus sp)*	228	Hemocyte lysomal membrane de-stabilization	(Canesi et al., 2007)
Zebra mussel *(Dreissena polymorpha)*	1000	↓ attachment and siphon extension	(Quinn et al., 2006)

Species	Exposure (µg/L)	Effect	Reference
Oyster *(Crassostrea gigas)*	1-100 µg/L	↓ sperm motility	(Nice, 2005)
Leptodea fragilis	570 (130)	24-hr LC50 (NOEC)	(Milam et al., 2005)
Lampsilis cardium	1190 (200)	24-hr LC50 (NOEC)	
Lampsilis siliquoidea	490 (240)	24-hr LC50 (NOEC)	
Megalonaias nervosa	560 (<180)	24-hr LC50 (NOEC)	
Ligumia subrostrata	1040 (240)	24-hr LC50 (NOEC)	
Utterbackia imbecillis	770 (340)	24-hr LC50 (NOEC)	
Pimphales promelas	136	96-hr LC50	(Teneyck and Markee, 2007)
Ceriodaphnia dubia	92.4	48-hr LC50	
Lumbriculus variegatus	6.3 µmol/L	LC50 water	(Maenpaa and Kukkonen, 2006)
	11.5 µmol/kg	LC50 body burden	
Lymnaea stagnalis	100	lesions in foot, mantle	(Czech et al., 2001)
Algae (unspecified)	80 - 530	Algal growth EC50	(Graff et al., 2003)
Algae *(Isochrysis galbana)*	1000	Absence of photosynthesis	(Correa-Reyes et al., 2007)
Diatom *(Skeletonema Costatum)*	27 µg/L.	EC50 growth	
Amphipod *(Eohaustorius estuaries)*	191	LC50	(Hecht and Boese, 2002)
	116	1-hr re-burial EC50	
Nematodes	1 mg/kg sediment	↓ is abundance of some species	(Hoss et al., 2004)